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or: Sirbasku

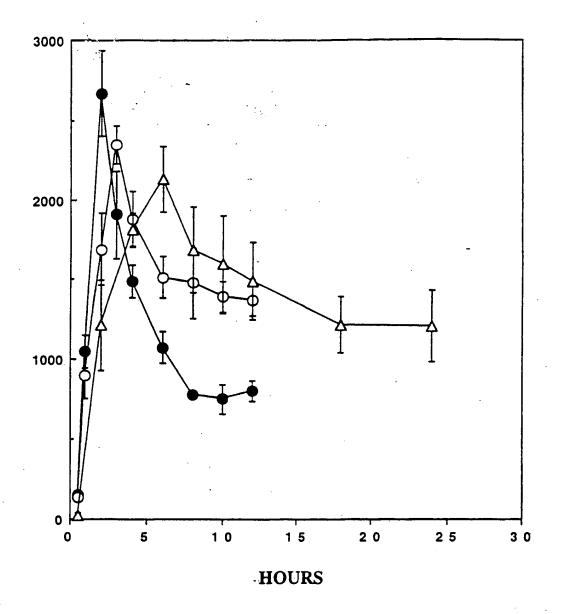
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· act: C.G. Mintz (713) 238-8000

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EFFECT OF TEMPERATURE ON THE SPECIFIC BINDING OF 5 nM ³H-E₂ TO MTW9/PL2 CELLS



The kinetics are shown (\pm SD of triplicates) at 37 $^{\circ}$ C (closed circles), 23 $^{\circ}$ C (open circles), and at 4 $^{\circ}$ C (open triangles).

Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 300,000 CPM and 1.0 x 10^6 cells.

BOUND/FREE



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Inventor: Sirbasku

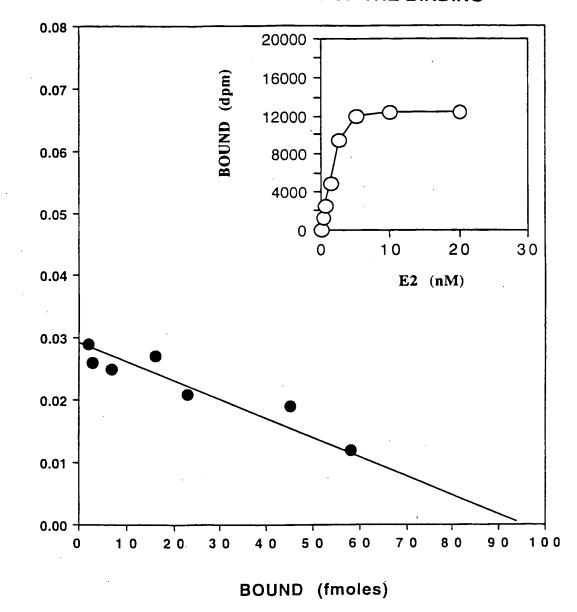
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FIGURE 2

EFFECT OF CONCENTRATION ON THE SPECIFIC BINDING OF ³H-E₂ TO MTW9/PL2 CELLS AND A SCATCHARD ANALYSIS OF THE BINDING



Scatchard analysis of $^3\text{H-E}_2$ binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and DES (100-fold excess) over the concentration range 37 pM to 5.0 nM $^3\text{H-E}_2$. In both experiments, 5 nM $^3\text{H-E}_2$ was 300,000 DPM. Each assay sample contained 1.0 x 10 6 cells.

INSERT: The insert shows a separate experiment in which the effect of ³H-E₂ concentration was measured on specific binding (DPM) after 2 h at 37 °C in phenol red-free D-MEM/F-12.



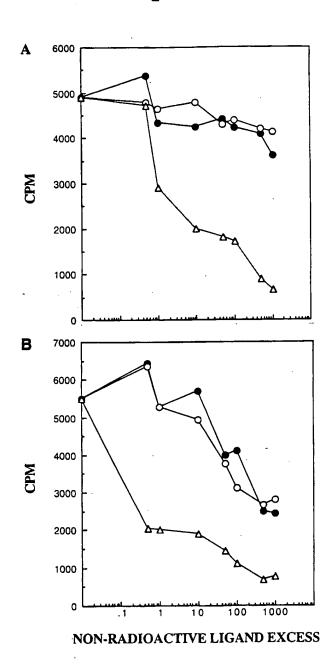
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Page 3 of 148 FIGURE 3

EFFECT OF OTHER STEROID HORMONES ON THE BINDING OF ³H-E₂ TO MTW9/PL2 CELLS



- (A) shows the effects of unlabeled DES (open triangles), unlabeled DHT (open circles), and unlabeled T (closed circles).
- (B) shows the effects of unlabeled DES (open triangles), unlabeled progesterone (open circles), and unlabeled cortisol (closed circles).



Inventor: Sirbasku

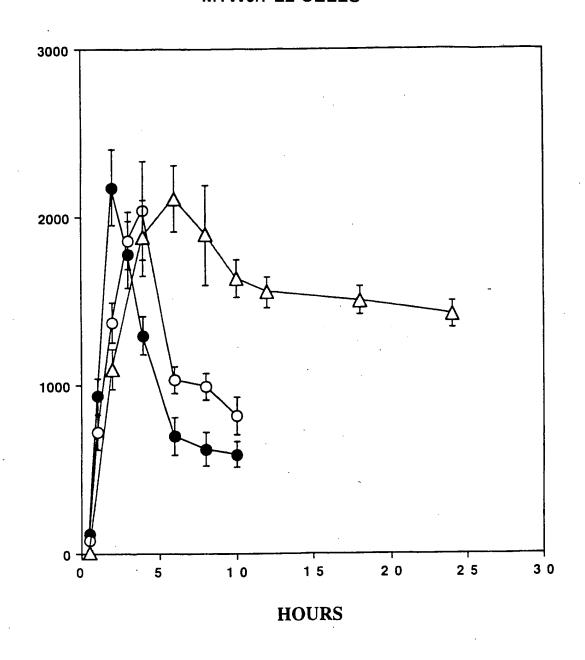
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FIGURE 4

EFFECT OF TEMPERATURE ON THE SPECIFIC BINDING OF 5 nM ³H-PROGESTERONE TO MTW9/PL2 CELLS



The kinetics are shown (SD of triplicates) at 37° C (closed circles), 23° C (open circles), and at 4° C (open triangles). Specific binding was determined in phenol red-free D-MEM/F-12. Each assay sample contained 287,000 CPM 3 H-progesterone and 1.0×10^{6} cells.



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Inventor: Sirbasku

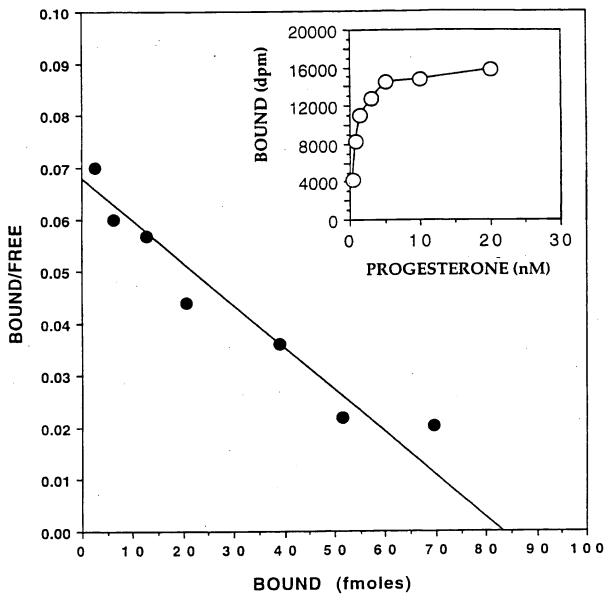
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FIGURE 5

EFFECT OF CONCENTRATION ON THE SPECIFIC BINDING OF ³H-PROGESTERONE TO MTW9/PL2 CELLS



A Scatchard analysis of 3 H-progesterone binding (closed circles) was conducted using the traditional method with labeled-unlabeled mixtures of hormone and R5020 (100 fold excess) over the concentration range 37 pM to 5.0 nM 3 H-progesterone. In both experiments, 5.0 nM 3 H-progesterone was 287,000 CPM. Each assay sample contained 1.0 x 10 6 cells.

INSERT: The insert shows a separate experiment in which the effect of ³H-progesterone concentration was measured on specific binding (bound dpm) after 2 h at 37° C in phenol red-free D-MEM/F-12.



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Inventor: Sirbasku

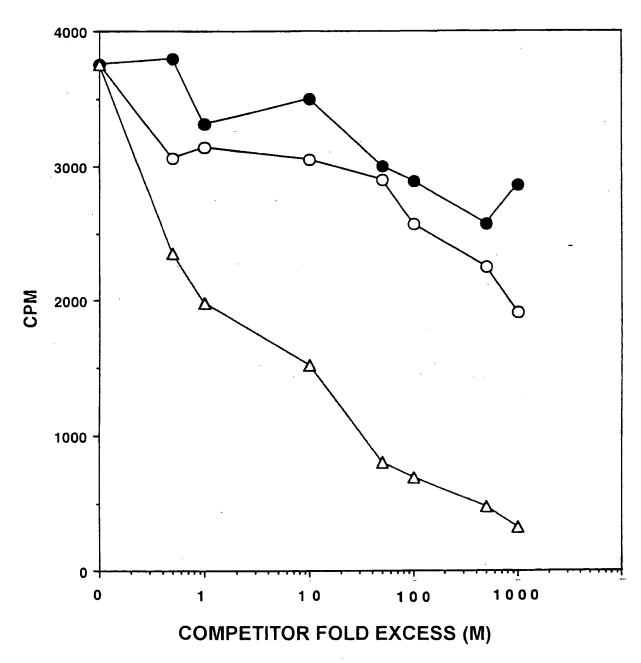
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FIGURE 6

EFFECT OF STEROID HORMONES ON THE BINDING OF ³ H-PROGESTERONE TO MTW9/PL2 CELLS



The cells were incubated at 37° C for 2 h in the presence of 5 nM 3 H-progesterone (287,000 CPM) alone or in the presence of the labeled hormone plus the designated fold excess (M) of unlabeled R5020 (open triangles), unlabeled DHT (open circles), or unlabeled T (closed circles). Each assay sample contained 1.0 x 10 6 cells.

Inventor: Sirbasku

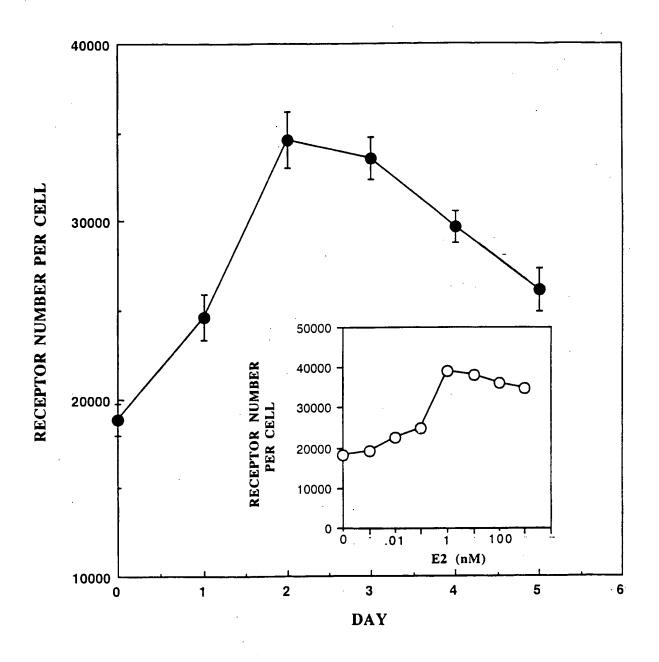
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EFFECT OF E 2 ON THE PROGESTERONE RECEPTOR **CONTENT OF MTW9/PL2 CELLS**



Each specific binding presented is the average of triplicate incubations ± SD (closed circles).

INSERT: The insert shows the effect of $\rm E_2$ concentration in the culture medium for 2 d prior to the assay of progesterone receptors (open circles).



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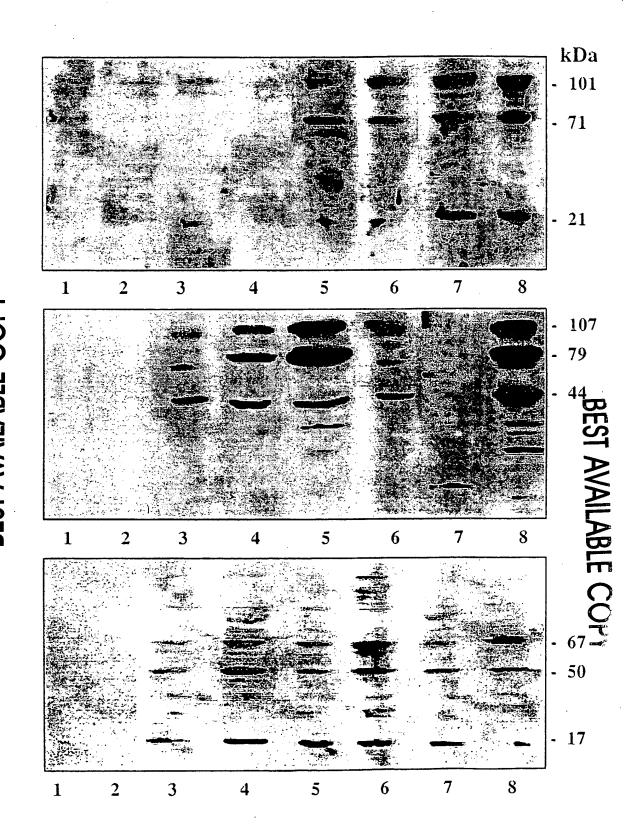
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WESTERN IMMUNOBLOTTING OF STEROID HORMONE RECEPTORS



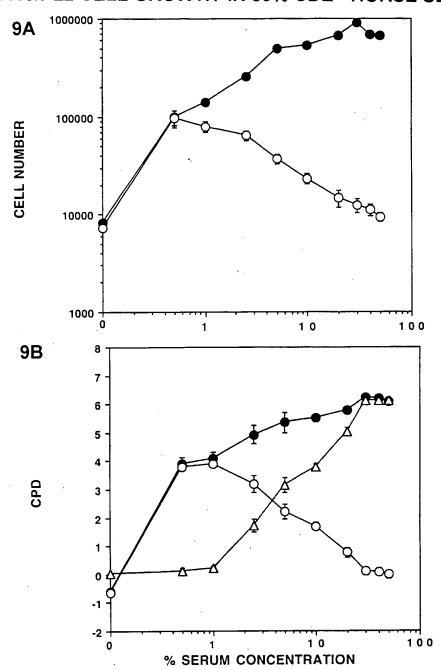
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FIGURE 9

MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM



- A: DATA EXPRESSED AS CELL NUMBER AFTER 7 DAYS Growth with 1.0 x 10 M E (closed circles) and without hormone (open circles) in medium containing the designated concentrations of serum.
- B. DATA IN (A) EXPRESSED AS CPD
 The symbols indicate the same conditions as (A)
 except the open triangles show CPD differences
 between growth in dishes with and without the
 hormone (Difference = estrogenic effect on growth).

Inventor: Sirbasku

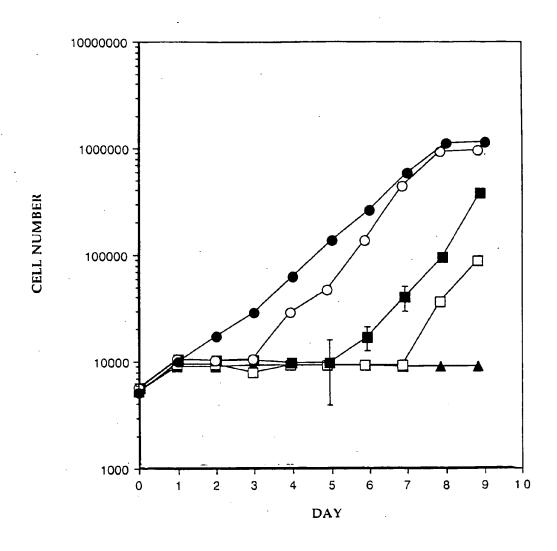
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MTW9/PL2 CELL GROWTH IN 50% CDE - HORSE SERUM WITH ESTROGENS ADDED AT VARIOUS TIMES AFTER SEEDING



LEGEND:

Control growth in the absence of exogenous estrogen is shown by (triangles). In other dishes, 1.0×10^{-8} M E₂ was added at the beginning of the experiment (closed circles), after 48 h (open circles), after 96 h (closed squares), or after 144 h (open squares).

Inventor: Sirbasku

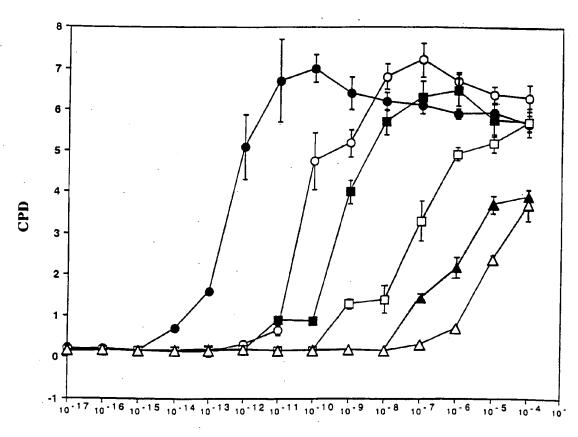
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FIGURE 11

STEROID HORMONE DOSE RESPONSE EFFECTS WITH MTW9/PL2 CELLS IN 50% CDE - HORSE SERUM



STEROID HORMONE (M)

LEGEND:

Closed circles = E₂
Open circles = E₁
Closed squares = E₃
Open squares = Progesterone
Closed triangles = DHT
Open triangles = T



Inventor: Sirbasku

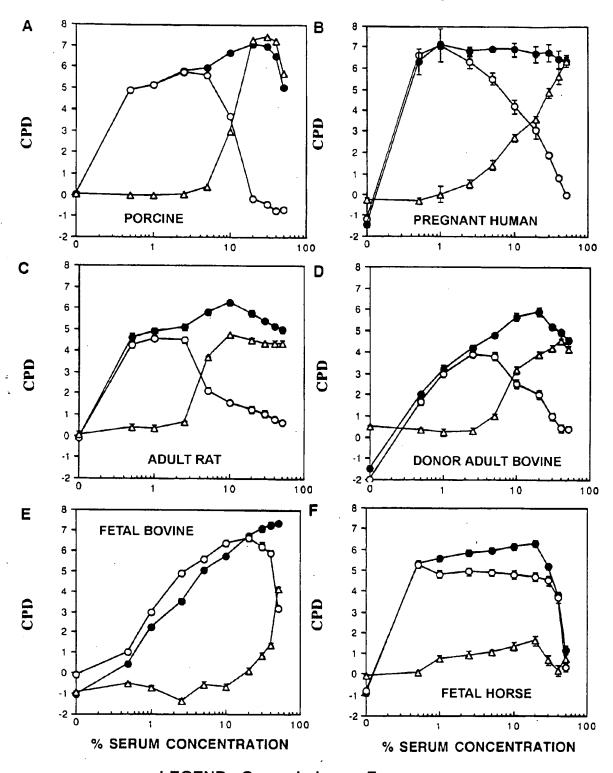
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FIGURE 12

MTW9PL2 CELL GROWTH IN CDE SERUM FROM DIFFERENT SPECIES



LEGEND: Open circles = -E₂
Closed circles = +E₂

Open triangles = Estrogenic effect

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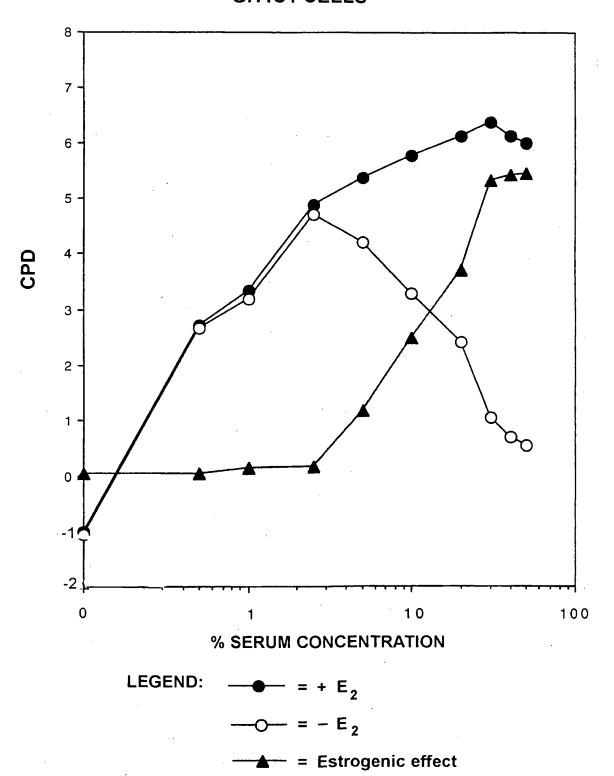
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FIGURE 13

CDE HORSE SERUM TITRATION GH4C1 CELLS



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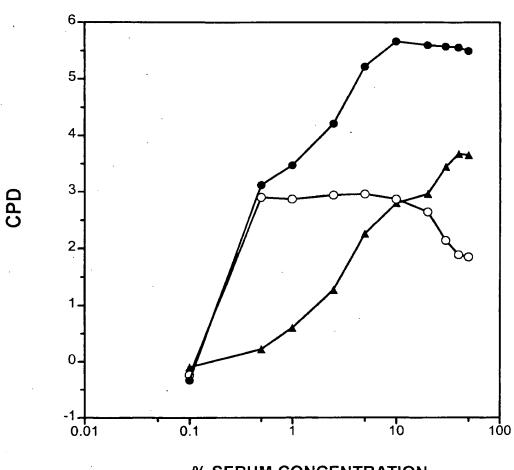
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FIGURE 14

ZR-75-1 CELLS IN CDE - HORSE SERUM ± 10 nM E2



% SERUM CONCENTRATION

LEGEND:

Closed circles = +E₂ Open circles = -E₂ Closed triangles = Estrogenic effect Con the test for

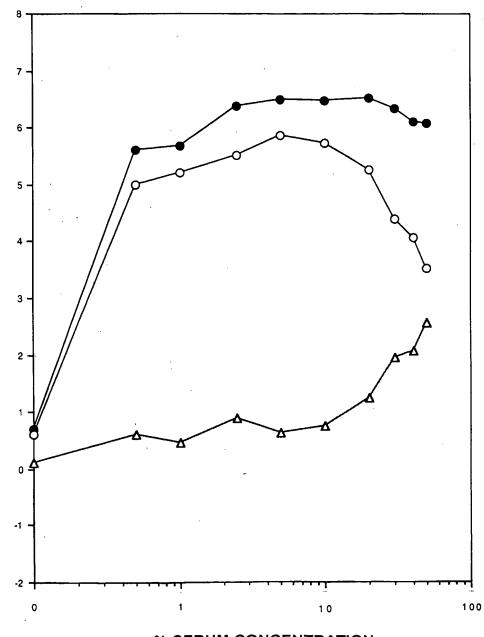
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-800

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FIGURE 15

MCF7A CELL GROWTH IN CDE - HORSE SERUM ± E2



% SERUM CONCENTRATION

LEGEND:

Closed circles = +E₂ Open circles = -E₂ Closed triangles = Estrogenic effect



Inventor: Sirbasku

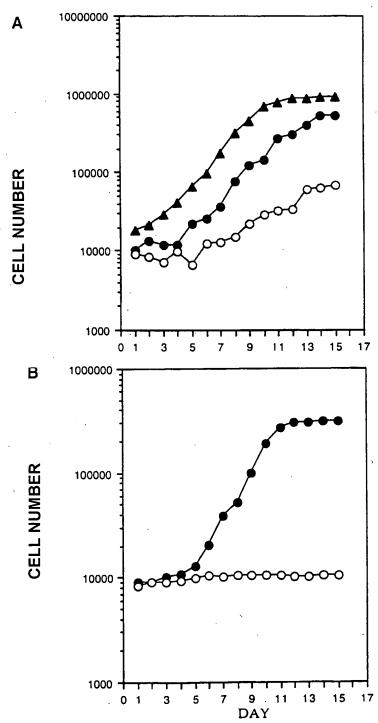
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FIGURE 16

GROWTH KINETICS OF T47D HUMAN BREAST CANCER CELLS IN CDE - HORSE SERUM ±10 nM E₂



- (A) The growth of the cells in medium with 20% (v/v) serum with 10 nM E_2 (closed circles) and without the steroid (open circles). As comparison, growth is shown in medium containing 10% (v/v) FBS (triangles).
- (B) T47D cell growth kinetics in medium with 50% (v/v) serum with E_2 (closed circles) and without the steroid (open circles).

Inventor: Sirbasku

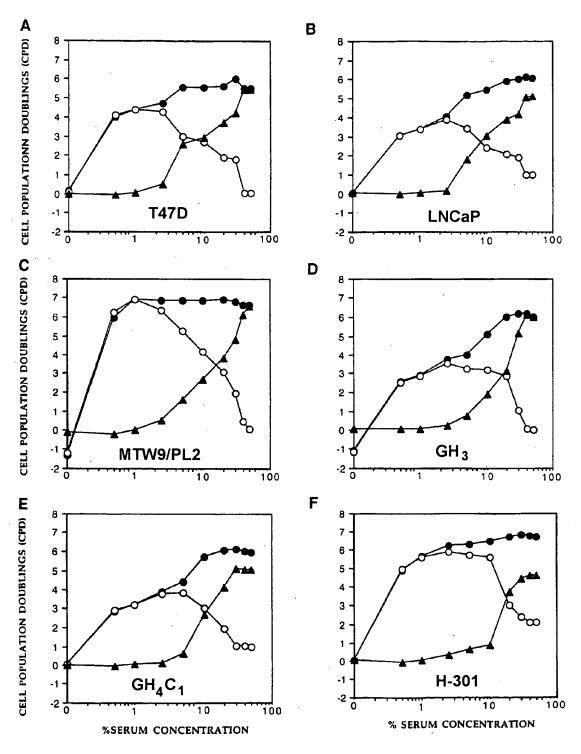
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LEGEND: Closed circles = Medium with 10 nM E_2 Open circles = Medium without E_2 Triangles = Estrogenic effect

Inventor: Sirbasku

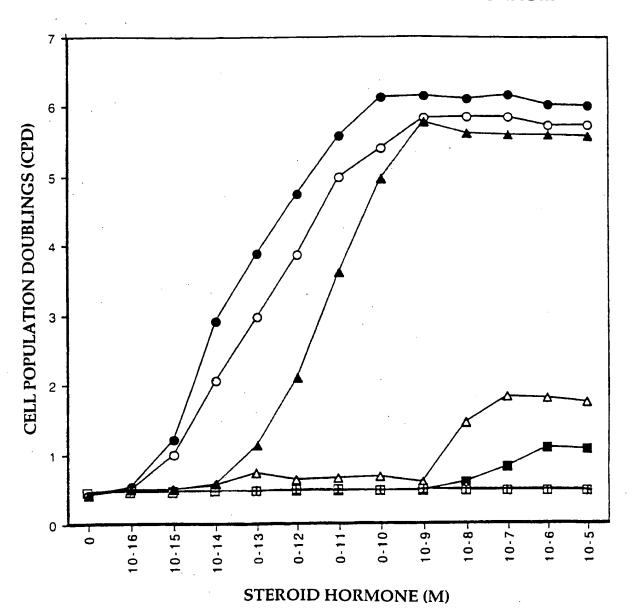
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FIGURE 18

DOSE RESPONSE OF STEROID HORMONES WITH T47D CELLS IN 50% CDE - HORSE SERUM



LEGEND:

Growth after 14 days is shown in response to:

Closed circles = E_2

Open circles = E_1

Closed triangles = E_3

Open triangles = DHT

Closed squares = Testosterone

Open squares = Progesterone

Crosses = Cortisol

Inventor: Sirbasku

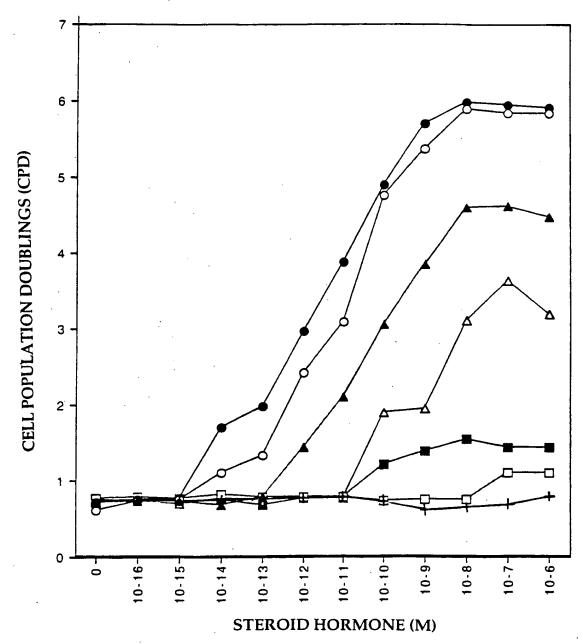
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FIGURE 19

DOSE RESPONSE OF STEROID HORMONES WITH $\mathrm{GH_4C_1}$ CELLS IN 50% CDE - HORSE SERUM



LEGEND:

Growth after 11 days is shown in response to:

Closed circles = E₂
Open circles = E₁
Closed triangles = E₃
Open triangles = DHT
Closed squares = Testosterone
Open squares = Progesterone
Crosses = Cortisol

Inventor: Sirbasku

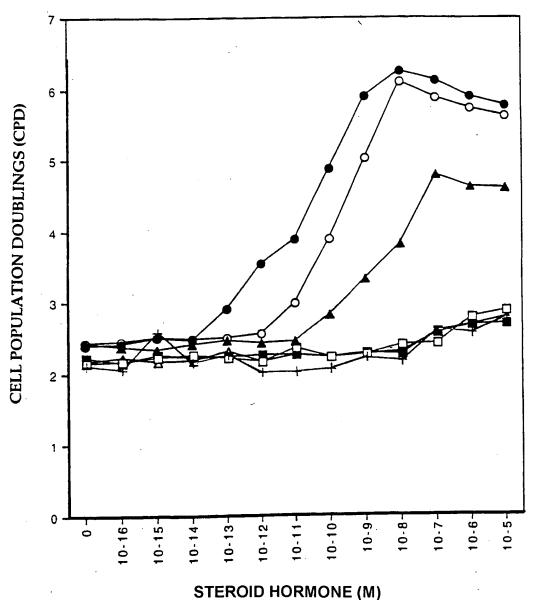
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FIGURE 20

DOSE RESPONSE OF STEROID HORMONES WITH H-301 CELLS IN 50% CDE - HORSE SERUM



LEGEND:

Growth after 9 days is shown in response to:

Closed circles = E_2

Open circles = E₁

Closed triangles = E₃

Open triangles = DHT

Closed squares = Testosterone

Open squares = Progesterone

Crosses = Cortisol

Inventor: Sirbasku

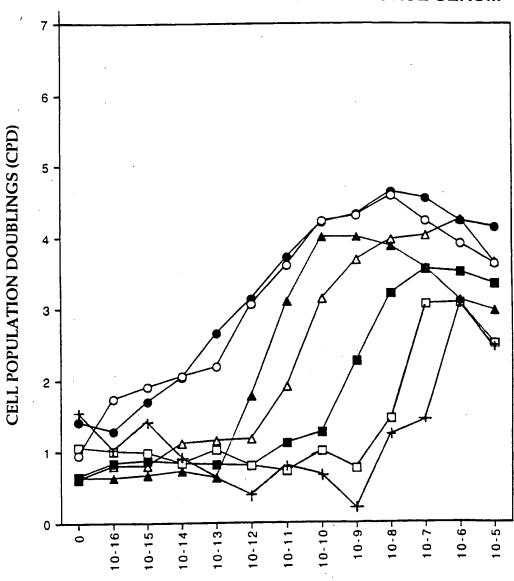
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FIGURE 21

DOSE RESPONSE OF STEROID HORMONES WITH LNCaP CELLS IN 50% CDE - HORSE SERUM



STEROID HORMONE (M)

LEGEND:

Growth after 14 days is shown in response to:

Closed circles = E₂

Open triangles = E_1

Open squares = E_3

Open circles = DHT

Closed triangles = Testosterone

Closed squares = Progesterone

Crosses = Cortisol

Inventor: Sirbasku

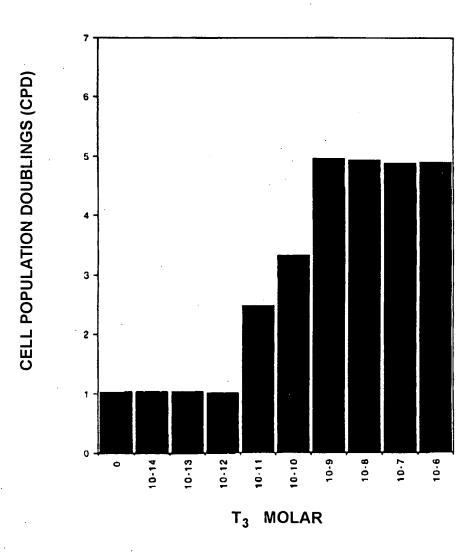
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FIGURE 22

T₃ TITRATION OF GH₃ CELLS GROWN IN SERUM - FREE MEDIUM (PCM)



Inventor: Sirbasku

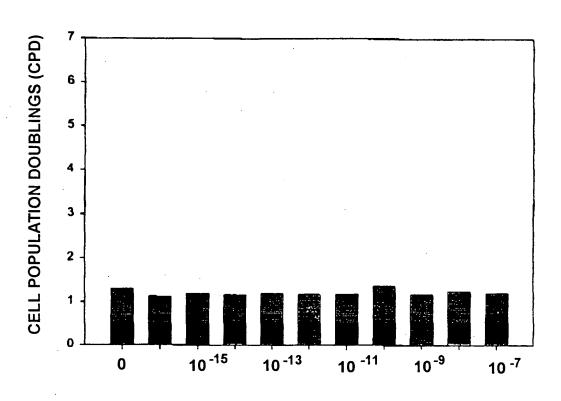
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${\sf E_2}$ TITRATION OF GH $_3$ CELLS GROWN IN SERUM-FREE MEDIUM MINUS T $_3$



E₂ MOLAR CONCENTRATIONS

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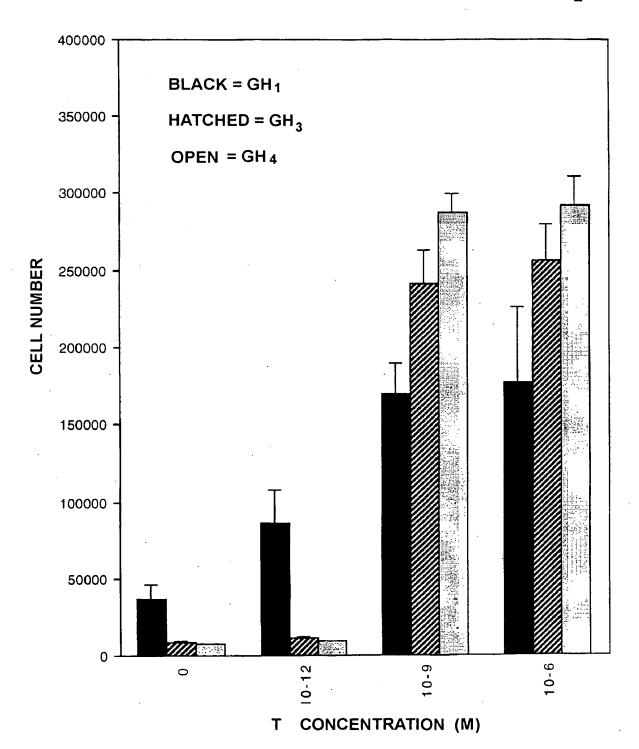
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FIGURE 24

EFFECT OF T_3 ON GH CELL LINES: GROWTH IN 2.5% CDE - HORSE SERUM WITH NO E_2





Inventor: Sirbasku

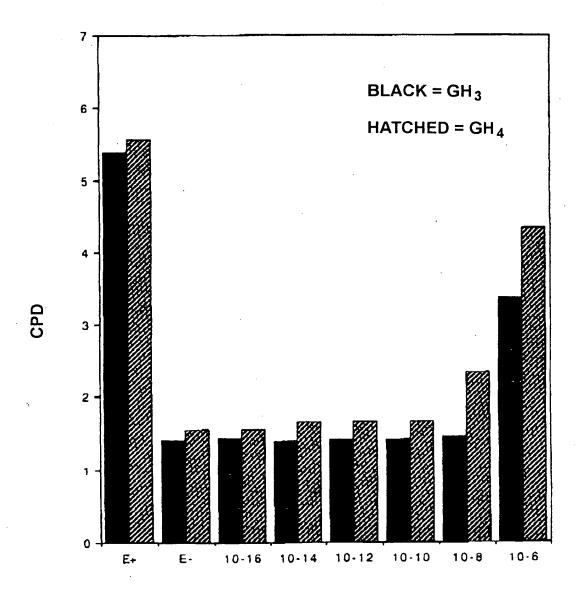
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FIGURE 25

EFFECT OF T₃ ON PITUITARY CELL LINES INCUBATED IN 50% CDE - HORSE SERUM



T₃ CONCENTRATION

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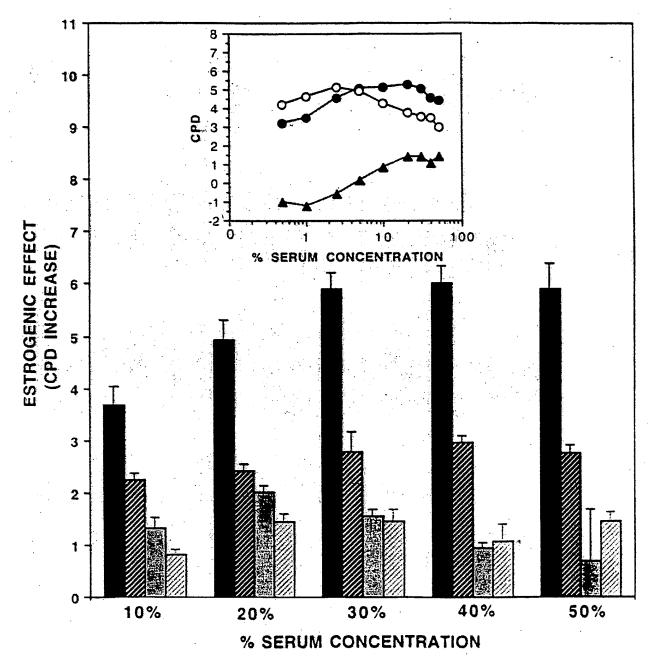
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COMPARISON OF 56°C AND 34°C CHARCOAL EXTRACTED SERUM



FILLED BARS: Estrogenic effect in 34°C prepared CDE-serum

DARK HATCHED BARS: 56°C prepared CDE-serum

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LIGHT SHADED BARS: Charcoal extracted at 34°C then charcoal extraction at 56°C LIGHT HATCHED BARS: Charcoal extracted at 34°C then incubation for 20 min at 56°C

INSERT: Dose-response growth effects of horse serum extracted at 34°C followed by incubation for 20 min at 56°C

Open circles - Growth without E_2 Closed circles - Growth with 1.0 x 10⁻⁸ M E_2 Triangles - Estrogenic effect

Inventor: Sirbasku

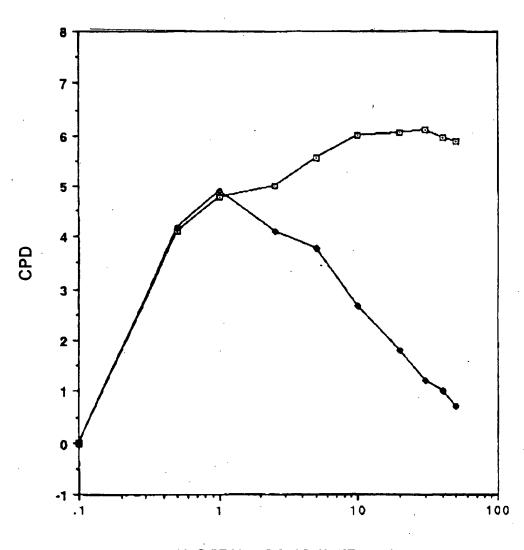
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FIGURE 27

HORSE SERUM TITRATION WITH MTW9/PL2 CELLS EXTRACTION BY XAD-4 RESIN



% SERUM CONCENTRATION

LEGEND:

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Open squares = + E₂

Closed squares = - E₂

Inventor: Sirbasku

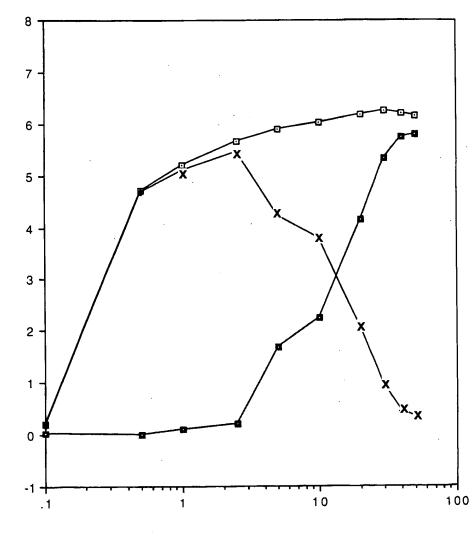
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FIGURE 28

HORSE SERUM TITRATION WITH T47D CELLS EXTRACTION BY XAD-4 RESIN



% SERUM CONCENTRATION

LEGEND:

1. 1.1 L.1 1.

Open squares = + E₂

 $XXX = -E_2$

Closed squares = Estrogenic effect

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Inventor: Sirbasku

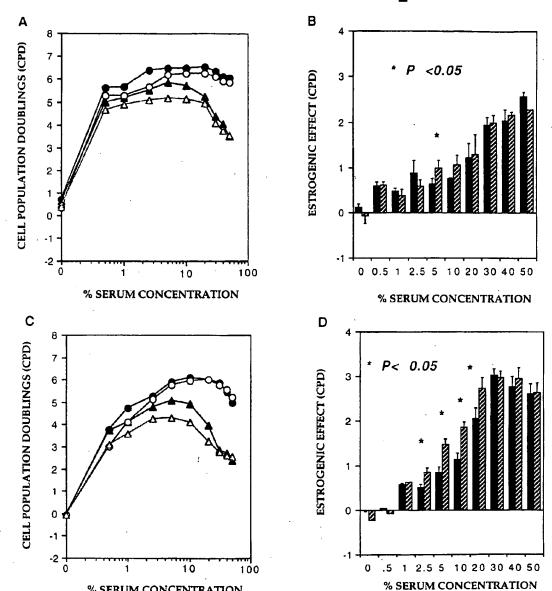
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FIGURE 29

MCF-7 CELL GROWTH IN CDE - HORSE SERUM # PHENOL RED AND # E2



LEGEND:

% SERUM CONCENTRATION

(A) MCF-7A cell growth in phenol red containing medium with E₂ (closed circles) and without E2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

(B) Estrogenic effects with MCF-7A cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E₂ minus the CPD in medium without added E2.

(C) MCF-7K cell growth in phenol red medium with E2 (closed circles) and without E2 (closed triangles), and in phenol red-free medium with E2 (open circles) and without E2 (open triangles).

(D) Estrogenic effects with MCF-7K cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

Inventor: Sirbasku

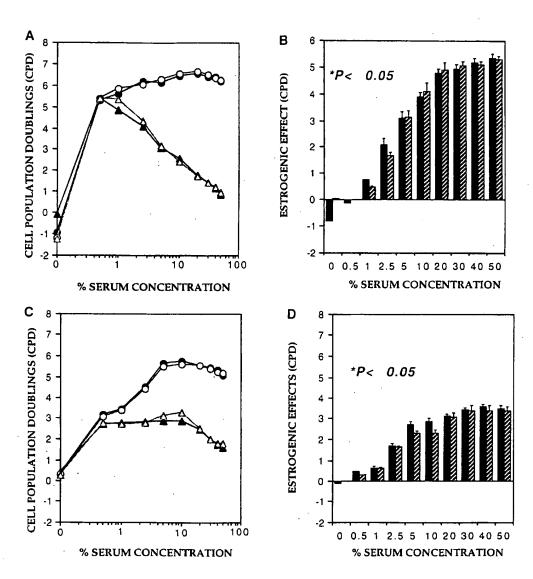
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FIGURE 30

T47D AND ZR-75-1 CELL GROWTH IN CDE-HS + PHENOL RED AND + E₂



LEGEND:

- (A) T47D cell growth in phenol red containing medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).
- (B) Estrogenic effects with T47D cells in medium with phenol red (solid bars) and without phenol red (shaded bars) were calculated from (A) and defined as the CPD in medium containing E_2 minus the CPD in medium without added E_2 .
- (C) ZR-75-1 cell growth in phenol red medium with $\rm E_2$ (closed circles) and without $\rm E_2$ (closed triangles), and in phenol red-free medium with $\rm E_2$ (open circles) and without $\rm E_2$ (open triangles).
- (D) Estrogenic effects with ZR-75-1 cells in medium with phenol red (solid bars) and without phenol red (shaded bars), calculated from (C).

Inventor: Sirbasku

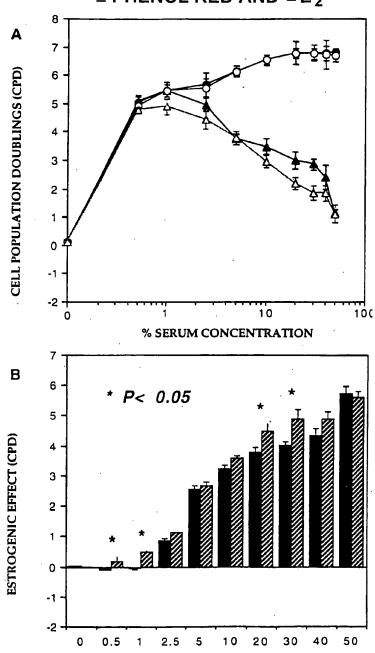
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FIGURE 31

MTW9/PL2 CELL GROWTH IN CDE - HORSE SERUM ± PHENOL RED AND ± E₂



LEGEND:

(A) MTW9/PL2 growth in phenol red medium with E_2 (closed circles) and without E_2 (closed triangles), and in phenol red-free medium with E_2 (open circles) and without E_2 (open triangles).

% SERUM CONCENTRATION

(B) Estrogenic effects with MTW9/PL2 cells in medium with phenol red (solid bars) and without (shaded bars) were calculated from (A).

Inventor: Sirbasku

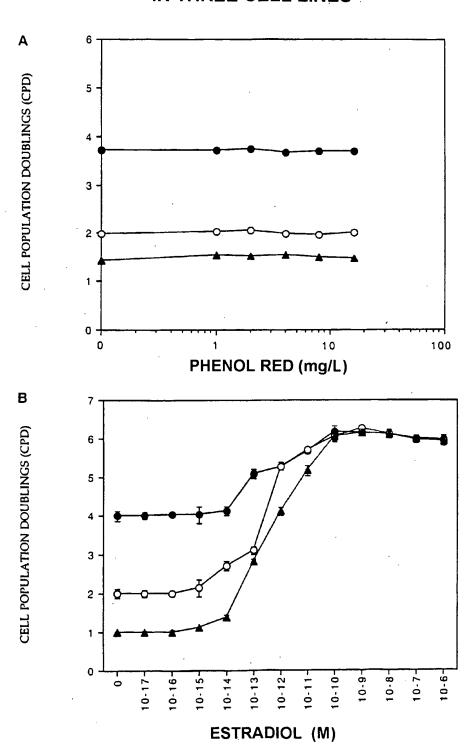
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FIGURE 32

DOSE RESPONSE TO PHENOL RED AND E₂ IN THREE CELL LINES



LEGEND: The growth of the MCF-7A (closed circles), MTW9/PL2 (open circles) and T47D (closed triangles) cell lines was assessed at 14, 7, and 12 days.

Inventor: Sirbasku

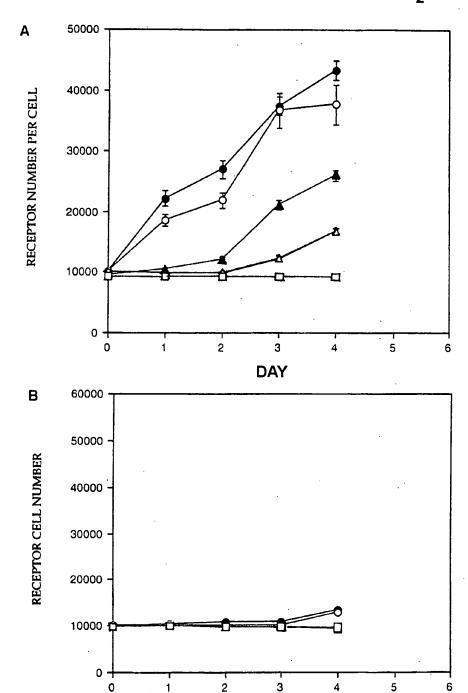
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FIGURE 33

PROGESTERONE RECEPTOR INDUCTION IN T47D CELLS BY PHENOL RED AND $\rm E_2$



LEGEND:

(A) The effects of E_2 at 1.0 x 10⁻⁸ M (closed circles), 1.0 x 10⁻¹⁰ M (open circles), 1.0 x 10⁻¹²M (closed triangles), 1.0 x 10⁻¹⁴ M (open triangles) and the control without added E_2 (open squares).

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(B) The effects of phenol red at 16 mg/L (closed circles), 8mg/L (open circles), 4 mg/L (closed triangles), 2 mg/L (open triangles), and the control without phenol red (open squares).

Inventor: Sirbasku

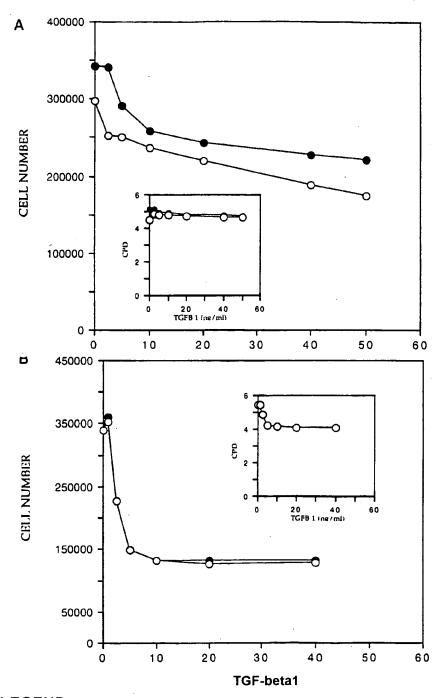
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EFFECT OF TGF-beta1 ON THE GROWTH OF BREAST/MAMMARY ORIGIN CELL LINES



LEGEND:

- (A) The effect of the transforming growth inhibitor on human breast MCF-7K cell growth as measured after 12 d either with 10 nM $\rm E_2$ (closed circles) or without the hormone (open circles). The insert shows conversion of the cell number results to CPD.
- (B) The same experiment with rat mammary MTW9/PL2 cells after 9 d growth.

Inventor: Sirbasku

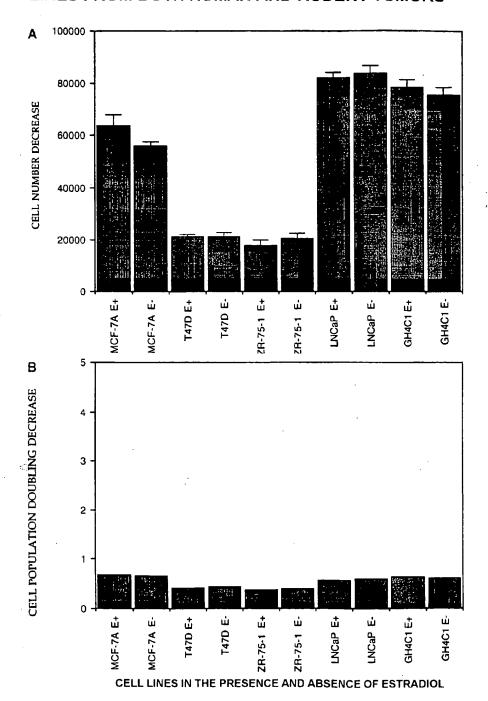
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EFFECT OF TGF-beta1 ON THE GROWTH OF CELL LINES FROM BOTH HUMAN AND RODENT TUMORS



In these studies, TGF-beta1 was added at 40 ng/ml. Estradiol (\pm E) indicates either no added E₂ or the steroid at 10 nM.

- (A) The effect of TGF-beta1 on five cell lines after 10-14 d growth in medium \pm E₂. The results are expressed as cell number decreases caused by TGF-beta1.
- (B) The CPD decreases caused by TGF-beta1 $\pm E_2$ with each of the cell lines shown in (A).

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Inventor: Sirbasku

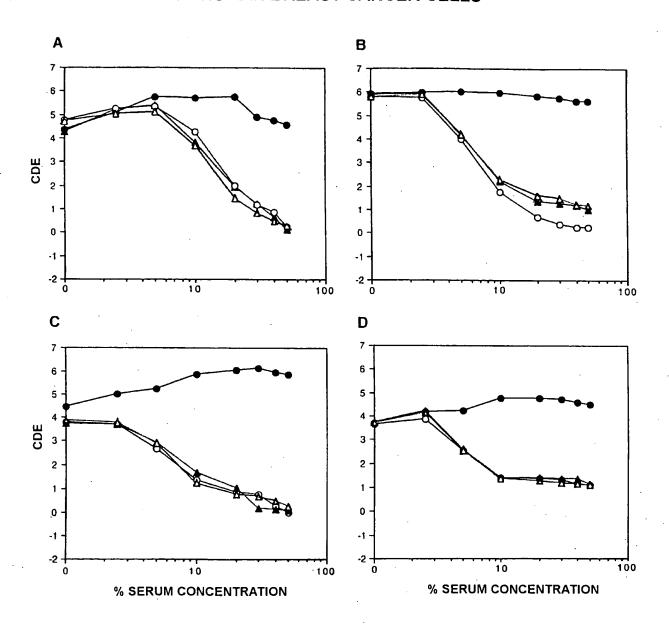
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FIGURE 36

EFFECT OF EGF AND TGF-alpha ON THE GROWTH OF HUMAN BREAST CANCER CELLS



The cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each line tested was grown in serum alone (open circles) and in serum plus 50 ng/ml EGF (open triangles), 50 ng/ml TGF-alpha (closed triangles), or 10 nM $\rm E_2$ without exogenous growth factors (closed circles). (A) - (D) show the results with the MCF-7A, MCF-7K, T47D, and ZR-75-1 cell lines, respectively.

Inventor: Sirbasku

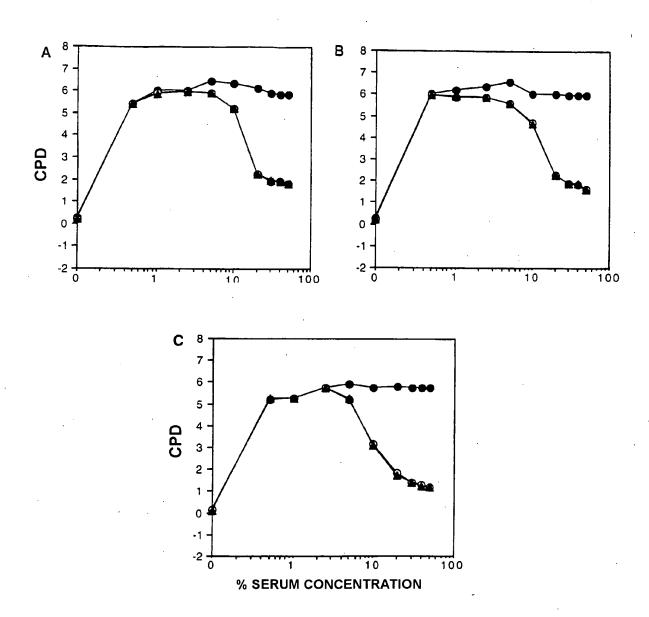
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FIGURE 37

EFFECT OF IGF-I ON THE GROWTH OF HUMAN BREAST CANCER CELLS



Breast cancer cells were grown in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum. Each cell line tested was grown in serum alone (open circles) and in serum plus 1.0 ug/ml IGF-I (triangles), or in serum with 10 nM E_2 without exogenous growth factors (closed circles). (A) - (C) show the results with the MCF-7K, MCF-7A and T47D cells, respectively. Assays were conducted for 12-14 d.

Express Mail EL818623541US Inventor: Sirbasku

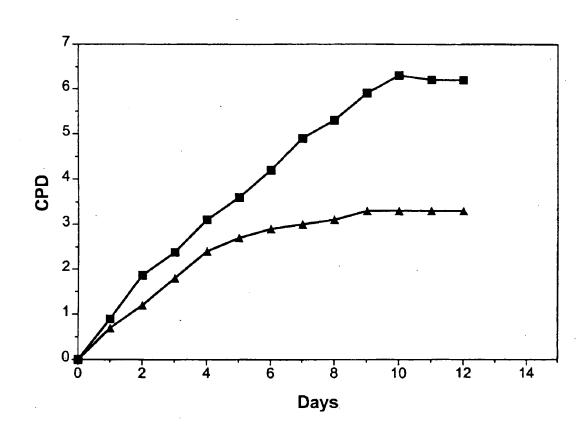
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FIGURE 38

T47D CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW FE" SERUM-FREE SERUM



LEGEND:

"STANDARD" MEDIUM

---- "LOW-FE" MEDIUM

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Inventor: Sirbasku

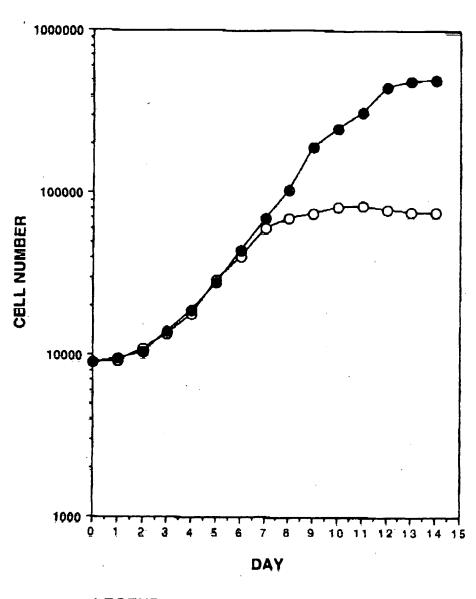
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FIGURE 39

LNCaP CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW-FE" SERUM-FREE MEDIUM



LEGEND:

"LOW-FE" MEDIUM

Express Mail EL818623541US Inventor: Sirbasku

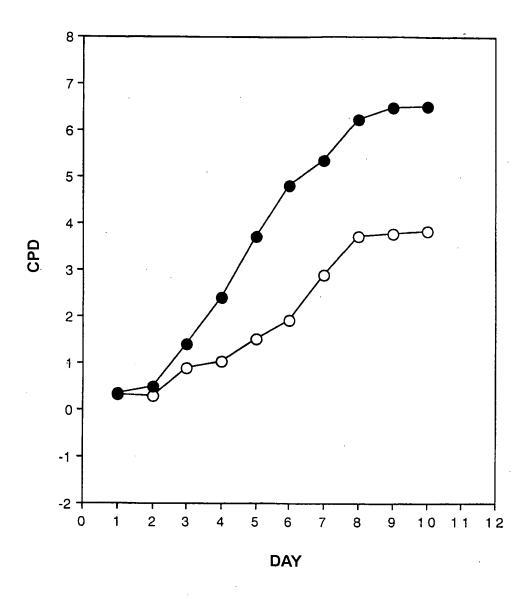
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FIGURE 40

MDCK CELLS IN STANDARD D-MEM/F-12 MEDIUM VS "LOW FE" SERUM-FREE MEDIUM



LEGEND:

-O- "STANDARD" MEDIUM

—● "LOW-FE" MEDIUM

Inventor: Sirbasku

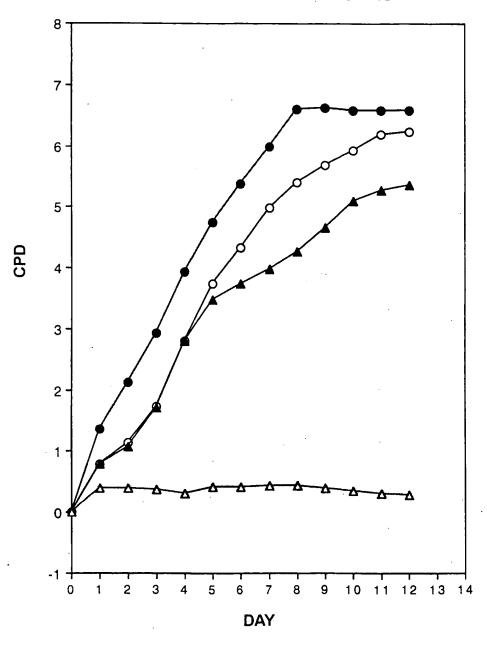
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LNCaP CELL GROWTH IN CAPM ± DHT AND 10% FETAL BOVINE SERUM



LEGEND:

Closed circles = Fetal bovine serum Open circles = CAPM + DHT Closed triangles = CAPM - DHT Open triangles = D-MEM/F12 only

Inventor: Sirbasku

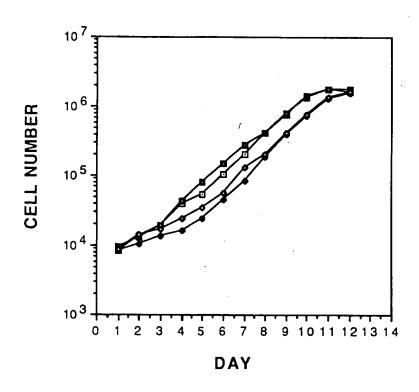
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FIGURE 42

PC3 AND DU145 GROWTH IN SERUM - FREE MEDIUM VS MEDIUM WITH 10% FETAL CALF SERUM



LEGEND:

→ → PC3 IN SERUM-FREE MEDIUM

DU145 IN SERUM-FREE MEDIUM

PC3 IN 10% FETAL CALF SERUM

DU145 IN 10% FETAL CALF SERUM



Inventor: Sirbasku

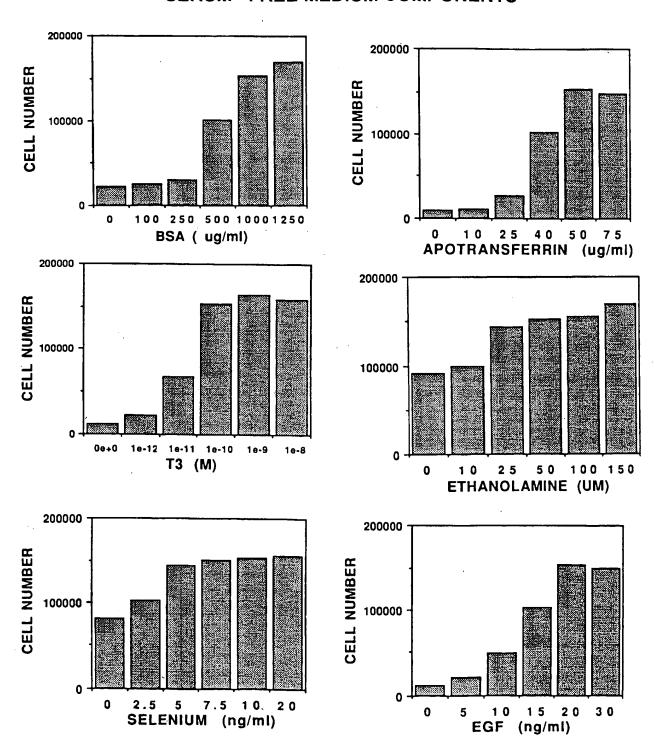
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FIGURE 43

DOSE RESPONSE EFFECTS OF CAPM SERUM - FREE MEDIUM COMPONENTS



Inventor: Sirbasku

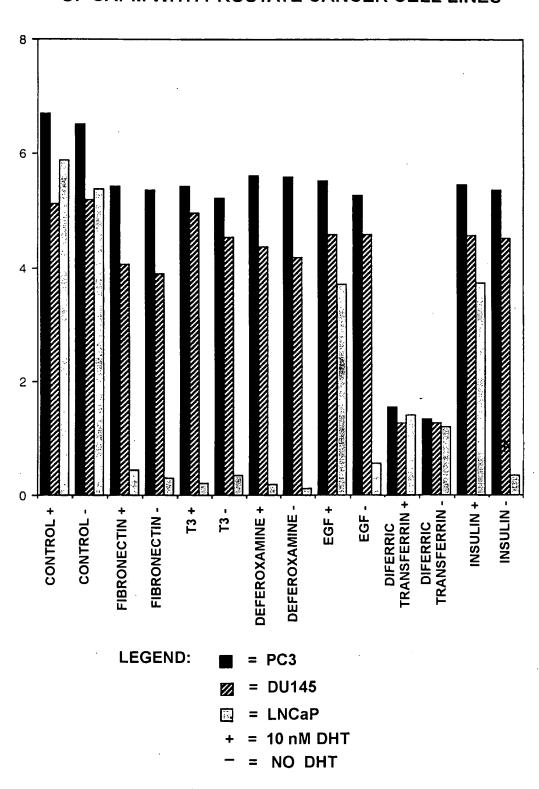
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FIGURE 44

DELETIONS OF INDIVIDUAL COMPONENTS OF CAPM WITH PROSTATE CANCER CELL LINES



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Inventor: Sirbasku

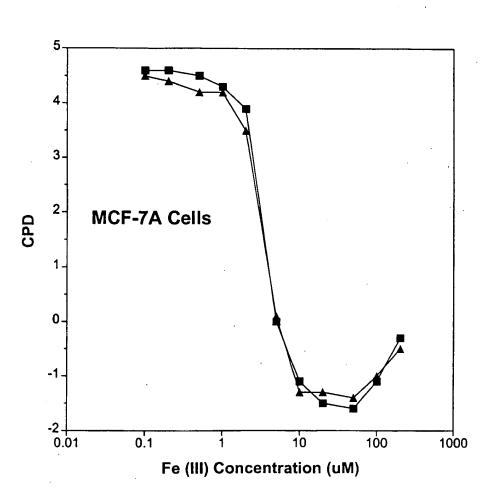
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FIGURE 45

EFFECT OF FE (III) IN MCF-7A CELL GROWTH IN DDM-2MF DEFINED MEDIUM



LEGEND:

-∎- plus E₂

minus E₂

Inventor: Sirbasku

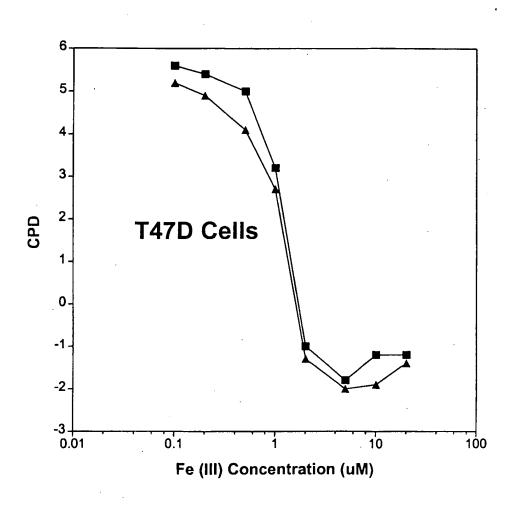
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FIGURE 46

EFFECT OF FE (III) IN T47D CELL GROWTH IN DDM-2MF DEFINED MEDIUM



LEGEND:

-- plus E₂

- minus E_2

Inventor: Sirbasku

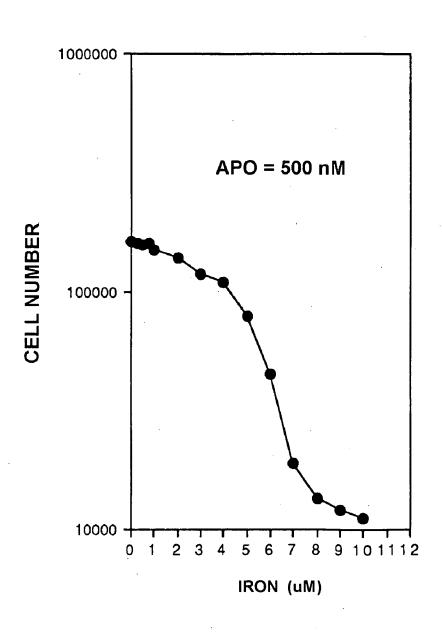
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FIGURE 47

EFFECTS OF INCREASING CONCENTRATIONS OF IRON ON LNCaP CELLS GROWN IN SERUM-FREE MEDIUM WITH APOTRANSFERRIN



Express Mail EL818623541US Inventor: Sirbasku

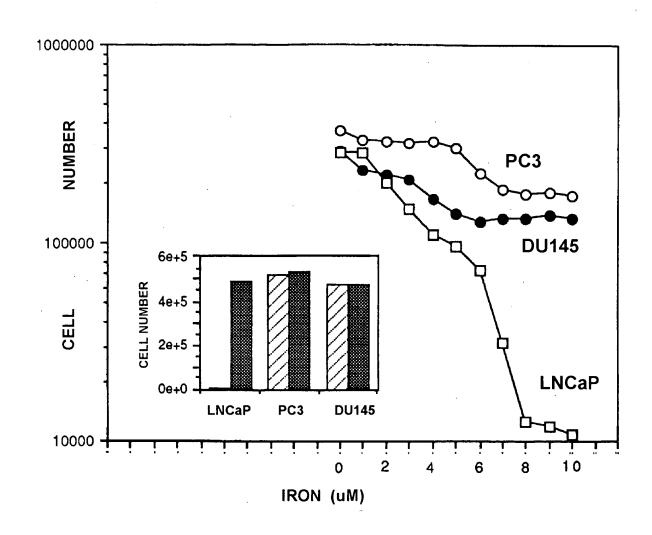
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FIGURE 48

EFFECTS OF IRON AND T₃ ON THREE PROSTATIC CELL LINES IN SERUM-FREE MEDIUM



INSERT:

DARK BARS = GROWTH IN CAPM PLUS T_3 LIGHT (HATCHED) BARS = GROWTH IN CAPM MINUS T_3

NOTE THE STRIKING DEPENDENCE OF LNCaP CELLS ON T₃

Inventor: Sirbasku

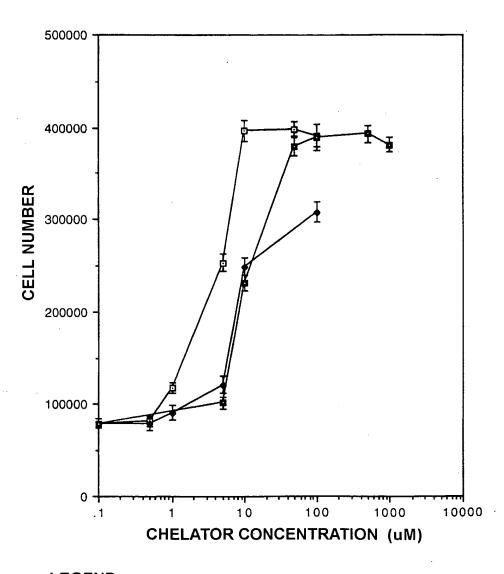
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FIGURE 49

GROWTH UNDER HIGH IRON CONDITIONS



LEGEND:

DEFEROXAMINE

→ EDTA

----- CITRATE

Inventor: Sirbasku

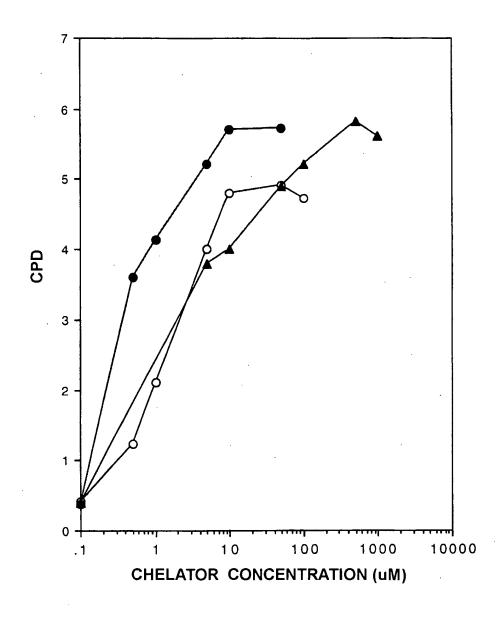
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FIGURE 50

EFFECT OF CHELATORS ON SERUM-FREE LNCaP GROWTH UNDER HIGH IRON CONDITIONS



LEGEND:

Closed circles = Deferoxamine

Open circles = Citrate

Closed triangles = EDTA

Inventor: Sirbasku

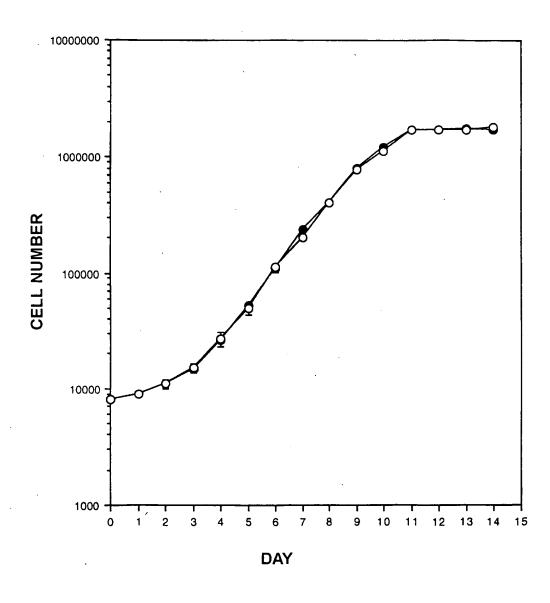
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FIGURE 51

DU145 GROWTH IN SERUM-FREE MEDIUM BASED ON "LOW FE" OR "STANDARD" MEDIUM



LEGEND:

Open circles = "Low Fe" medium

Closed circles = "Standard" medium

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Inventor: Sirbasku

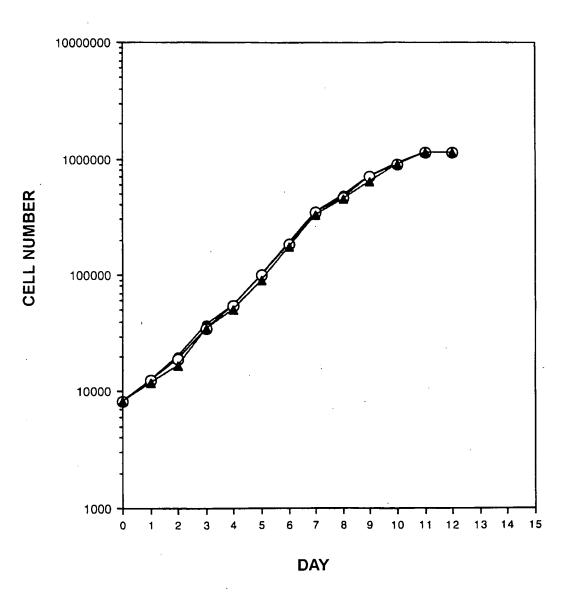
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FIGURE 52

PC3 GROWTH IN SERUM-FREE MEDIUM BASED ON "LOW FE" OR "STANDARD" MEDIUM



LEGEND:

Open circles = "Low Fe" medium

Closed triangles = "Standard" medium

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Inventor: Sirbasku

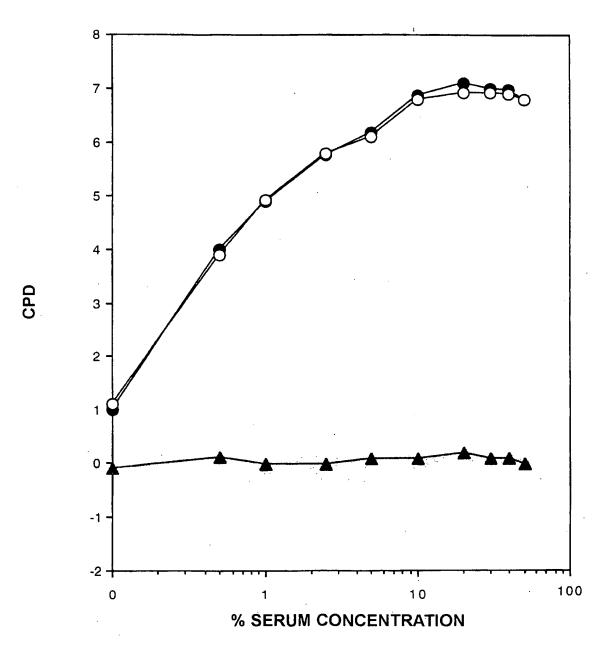
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FIGURE 53

CDE HORSE SERUM TITRATION ON DU145 CELLS



LEGEND:

+ 10 nM DHT

= STEROID FREE

- = ANDROGENIC EFFECT

Inventor: Sirbasku

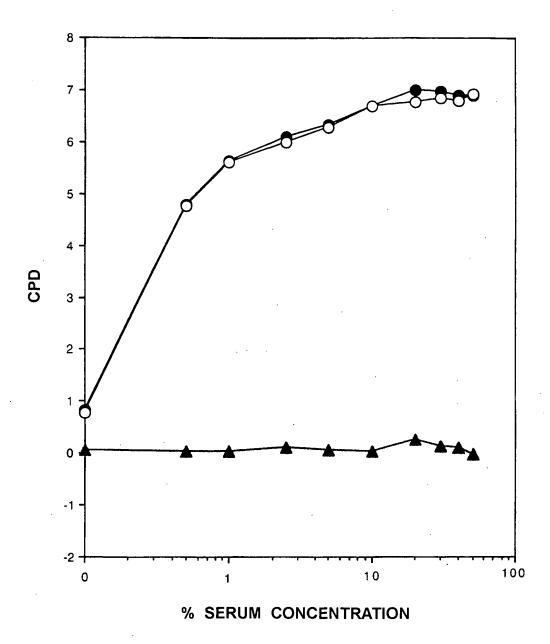
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CDE HORSE SERUM TITRATION ON PC3 CELLS



LEGEND:

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--- = + 10 nM DHT

—O— = STEROID FREE

= ANDROGENIC EFFECT

Inventor: Sirbasku

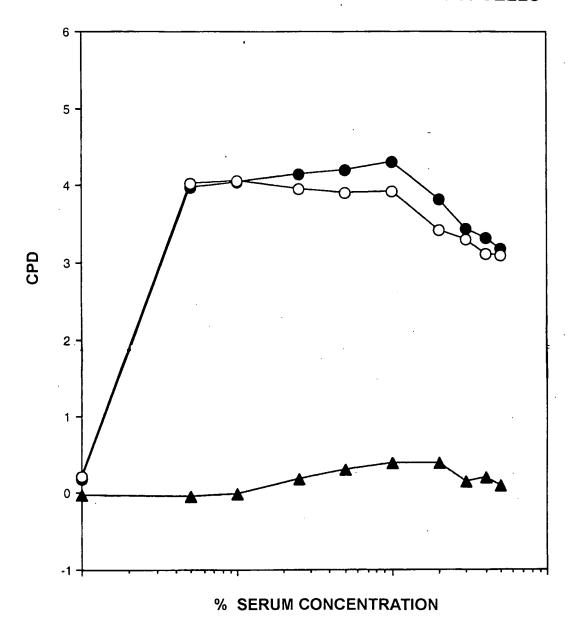
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FIGURE 55

CDE HORSE SERUM TITRATION ON ALVA-41 CELLS



LEGEND:

— = + 10 nM DHT

-O- = STEROID FREE

= ANDROGENIC EFFECT

Inventor: Sirbasku

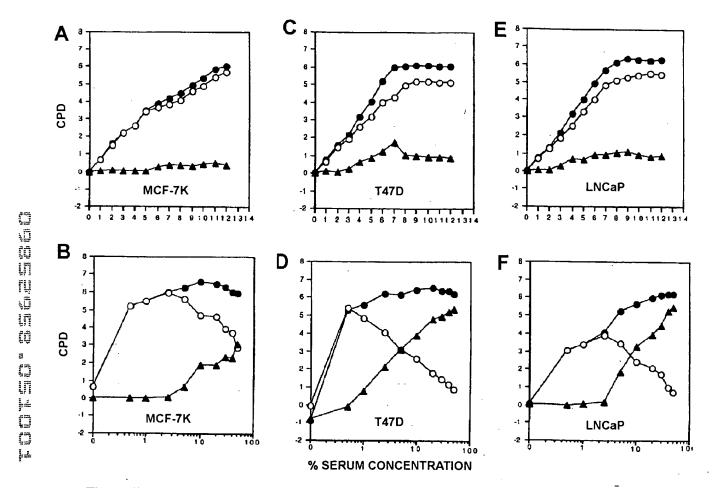
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FIGURE 56

EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE HUMAN TUMOR CELL GROWTH



The cells were grown in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) MCF-7K cell growth was measured daily in serum-free defined DDM-2MF with 10 nM E_2 (closed circles) and without steroid (open circles) E_2 . Triangles = estrogenic effect. (B) MCF-7K cell growth measured after 12 d in D-MEM-F-12 supplemented with the designated concentrations of serum with E_2 (closed circles) and without steroid (open circles). The estrogenic effect is shown by triangles. (C) and (D) show the same experiments as in (A) and (B), respectively, except with T47D cells.

(E) and (F) show the same experiments as in (A) and (B), respectively, except with LNCaP cells. In (E) the serum-free medium was CAPM.



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Inventor: Sirbasku

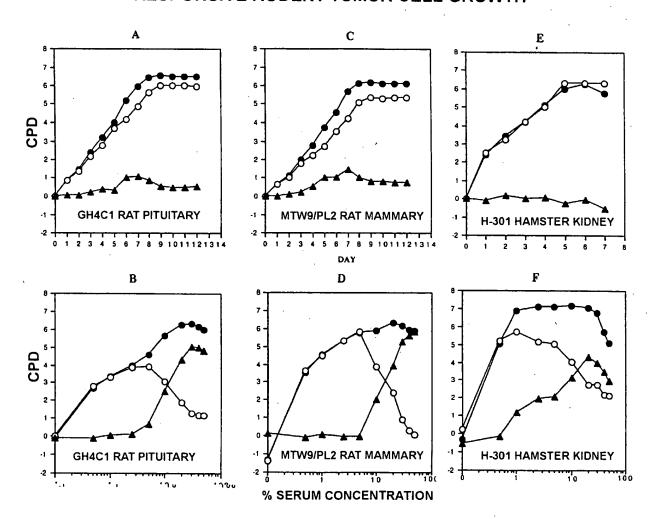
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EFFECTS OF ESTROGEN ON STEROID HORMONE-RESPONSIVE RODENT TUMOR CELL GROWTH



Comparison of the effects of estrogen on steroid hormone-responsive rodent tumor cell growth in serum-free defined medium and in D-MEM/F-12 supplemented with increasing concentrations of CDE horse serum.

(A) GH_4C_1 rat pituitary tumor cell growth measured daily in serum-free PCM-9 with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles. (B) GH_4 C_1 cell growth measured after 9 d in D-MEM-F-12 supplemented with the designated concentrations of CDE horse serum with E_2 (closed circles) and without E_2 (open circles). The estrogenic effect is shown by triangles. (C) and (D) show the same experiments as in (A) and (B) respectively, but with the MTW9/PL2 rat mammary tumor cells. The serum-free medium in (D) was DDM-2A. (E) and (F) show the same experiments as in (A) and (B), respectively, except with the H-301 hamster kidney tumor cells. In (E) the serum-free medium was CAPM.

Inventor: Sirbasku

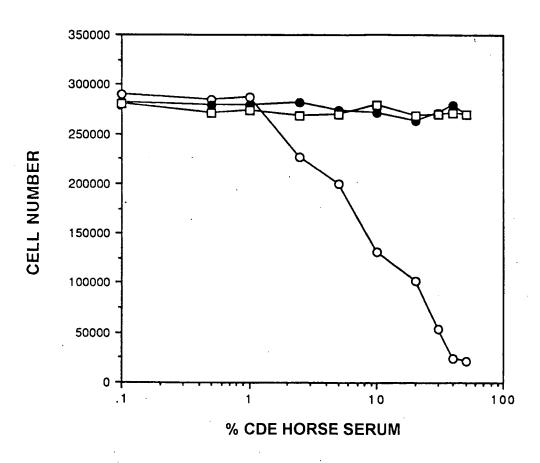
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FIGURE 58

CDE HORSE SERUM TITRATION ON LNCaP GROWTH IN SERUM FREE CONDITIONS



Inventor: Sirbasku

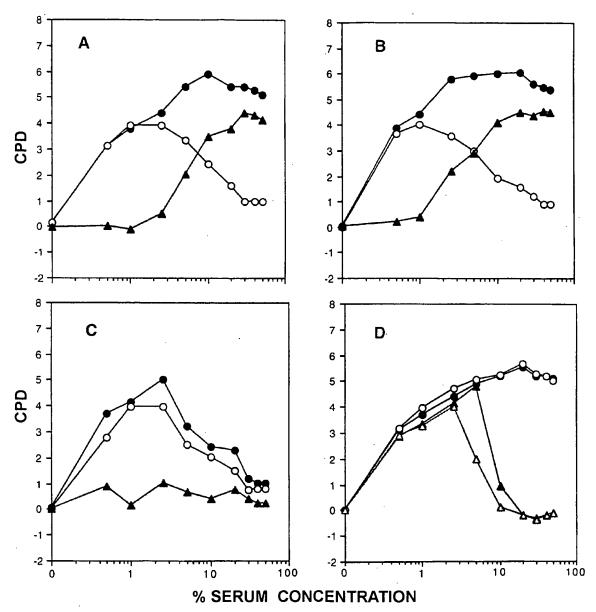
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FIGURE 59

THE EFFECT OF DHT, E₂, AND DES ON LNCaP CELLS GROWN IN CDE HORSE SERUM



- (A) Open circles = DHT
 Closed circles = + DHT
 Closed trianges = Androgenic effect
- (B) Open circles = E₂
 Closed circles = + E₂
 Closed triangles = Estrogenic effect
- (C) Open circles = DES
 Closed circles = + DES
 Closed triangles = Estrogenic effect
- (D) Open circles = DHT & DES Closed circles = E₂ & DES Open triangles = No additions Closed triangles = DES only

Inventor: Sirbasku

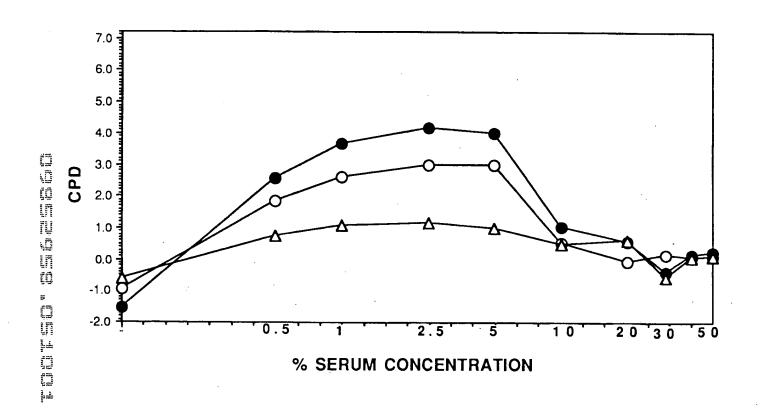
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FIGURE 60

TRIS DIALYSIS OF CDE HORSE SERUM AND ASSAY WITH MTW9/PL2 CELLS



$$\begin{array}{rcl}
 & = & + & E_2 \\
 & = & - & E_2 \\
 & & = & Estrogenic effect
\end{array}$$

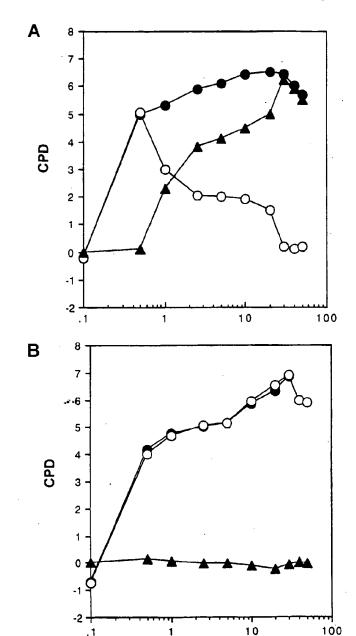
Inventor: Sirbasku Atty Dkt. No. 1944-00201

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FIGURE 61

ULTRAFILTRATION OF CDE HORSE SERUM AND ESTROGENIC EFFECTS WITH MTW9/PL2 CELLS



LEGEND:

(A) RETENTATE FROM AMICON MEMBRANE

% SERUM

(B) FILTRATE FROM AMICON MEMBRANE

Open circles = - E₂ Closed circles = + E₂

Inventor: Sirbasku Atty Dkt. No. 1944-00201

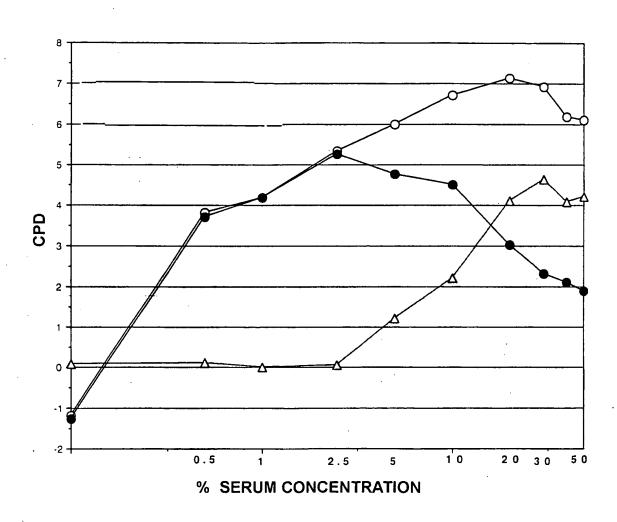
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FIGURE 62

CDE HORSE SERUM TREATED AT 50° C FOR 30 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

—△— = Estrogenic effect

Inventor: Sirbasku

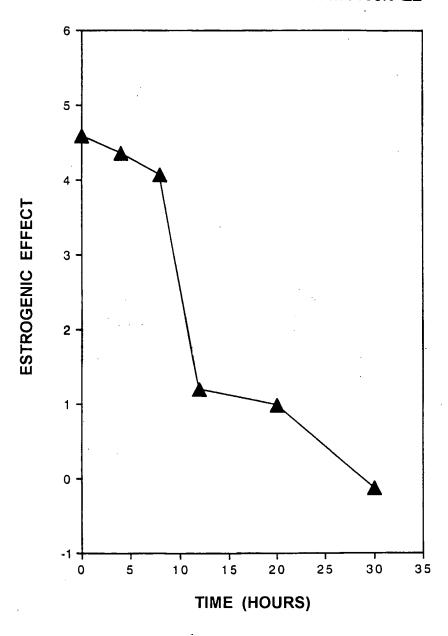
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FIGURE 63

EFFECT OF 50°C INCUBATION ON **ESTROGENIC EFFECT WITH MTW9/PL2**



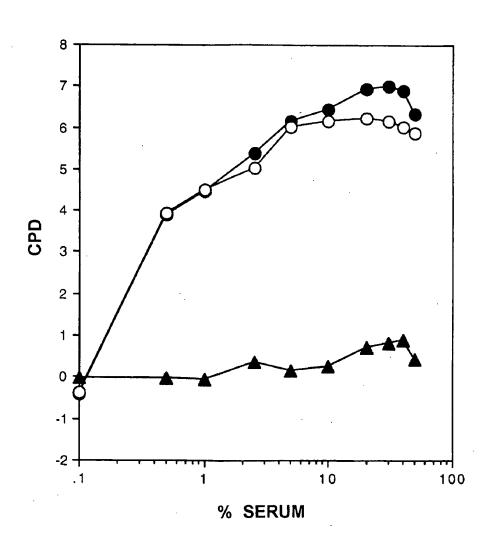
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FIGURE 64

CDE HORSE SERUM INCUBATION AT 50° C FOR 20 HOURS AND ASSAYED WITH MTW9/PL2



LEGEND:

Open circles = $-E_2$

Closed circles = $+ E_2$

Inventor: Sirbasku

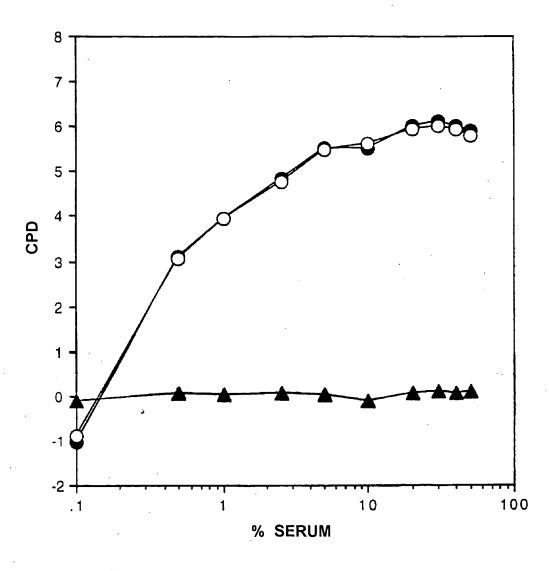
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FIGURE 65

CDE HORSE SERUM INCUBATED AT 60° C FOR 90 MINUTES AND ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

Open circles $= - E_2$

Closed circles = $+ E_2$

Closed triangles = Estrogenic effect

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Inventor: Sirbasku

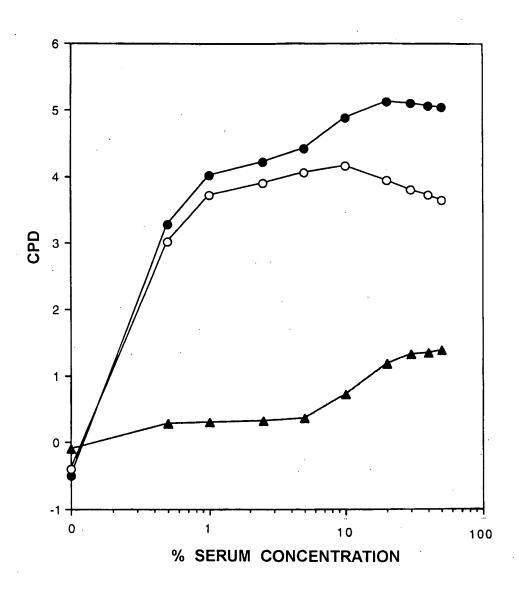
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FIGURE 66

AFFI-GEL BLUE TREATMENT OF CDE HORSE SERUM AND ASSAY WITH MTW9/PL2 CELLS



LEGEND:

Open circles = $-E_2$

Closed circles = $+ E_2$

Closed triangles = Estrogenic effect

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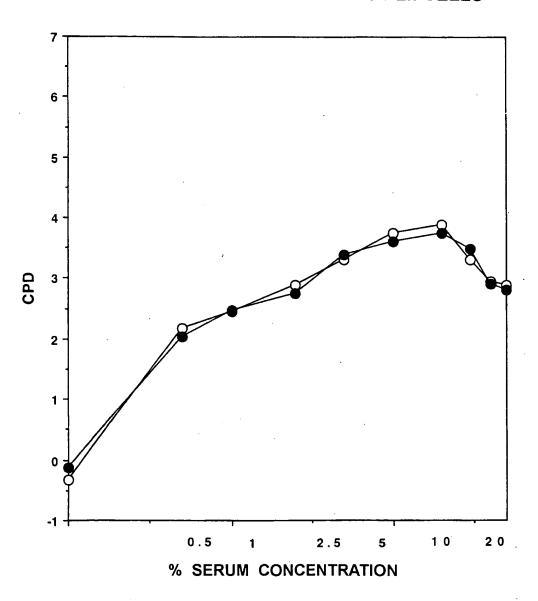
Inventor: Sirbasku Atty Dkt. No. 1944-00201

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DIALYSIS OF CDE HORSE SERUM AGAINST 6M UREA AND ASSAY WITH MTW9/PL2 CELLS



Inventor: Sirbasku

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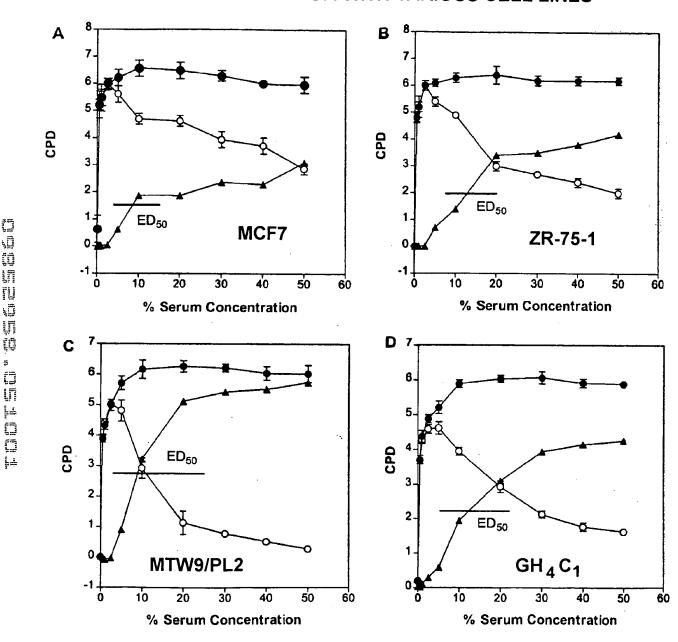
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FIGURE 68

ED_{50} MEASUREMENTS OF THE ESTROGENIC EFFECTS OF CDE HORSE SERUM WITH VARIOUS CELL LINES



LEGEND:

NO

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Closed circles = $+ E_2$

Open circles = $-E_2$

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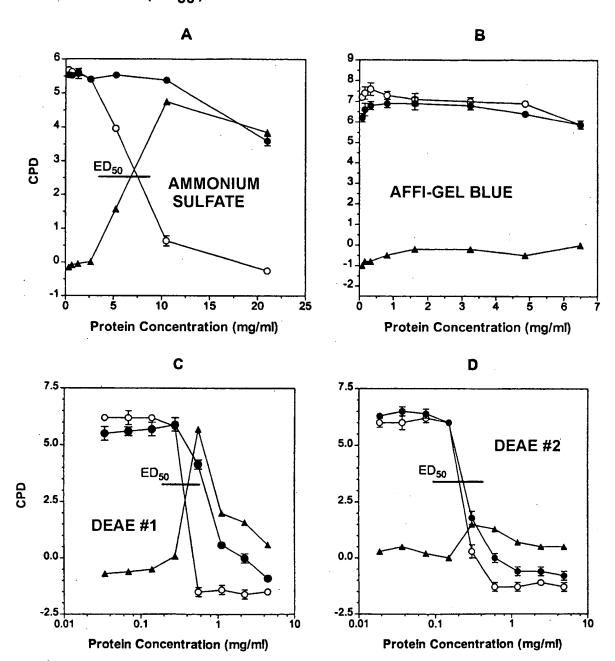
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ASSAY OF ESTROGENIC ACTIVITY (ED_{50}) OF CHROMATOGRAPHIC POOLS



LEGEND:

Closed circles = + E₂

Open circles = $-E_2$

Inventor: Sirbasku

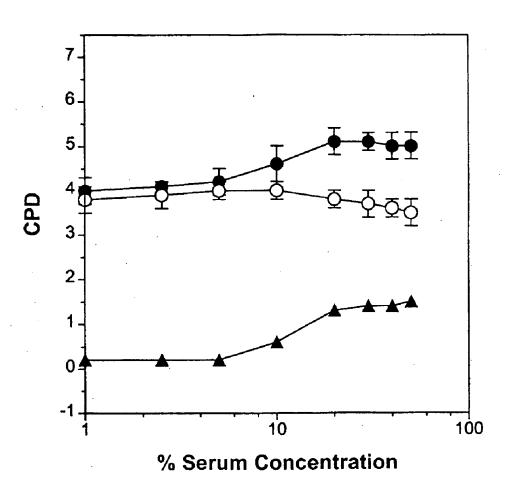
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FIGURE 70

AFFI-GEL BLUE BYPASS FRACTION ASSAYED WITH MTW9/PL2 CELLS



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$



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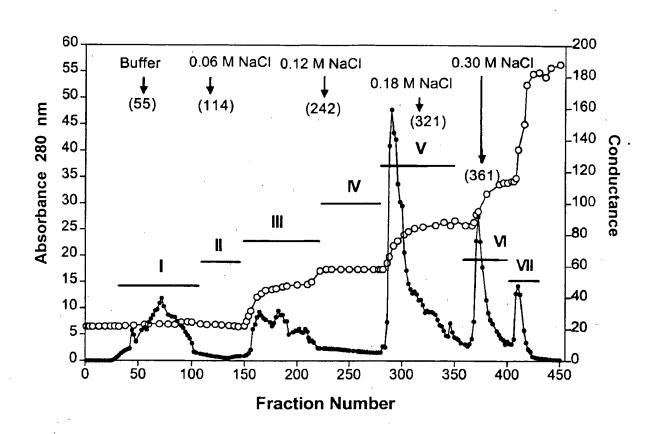
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FIGURE 71

DEAE SEPHAROSE CHROMATOGRAPHY OF CDE HORSE SERUM



LEGEND:

BARS = FRACTION POOLS

ARROWS = BUFFER CHANGES

Closed circles = Absorbance at 280 nm

Open circles = Conductance

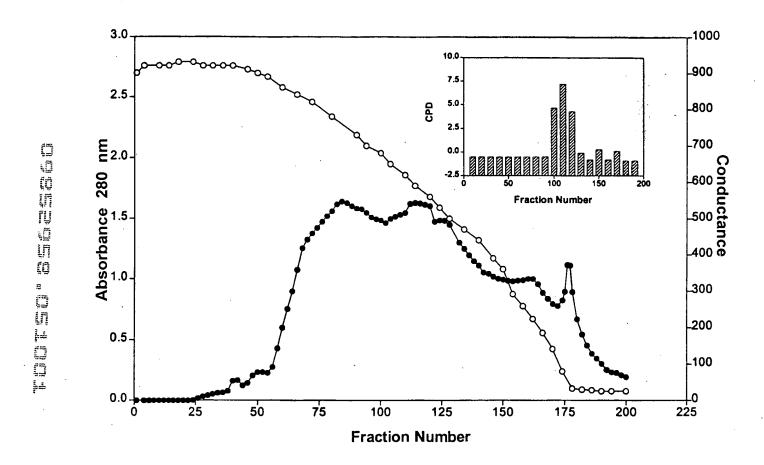
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 72

THE ELUTION PROFILE OF PHENYL SEPHAROSE WITH THE DEAE IV POOL



INSERT: Estrogenic activity with MTW9/PL2

LEGEND:

Closed circles = Absorbance 280 nm

Open circles = Conductance

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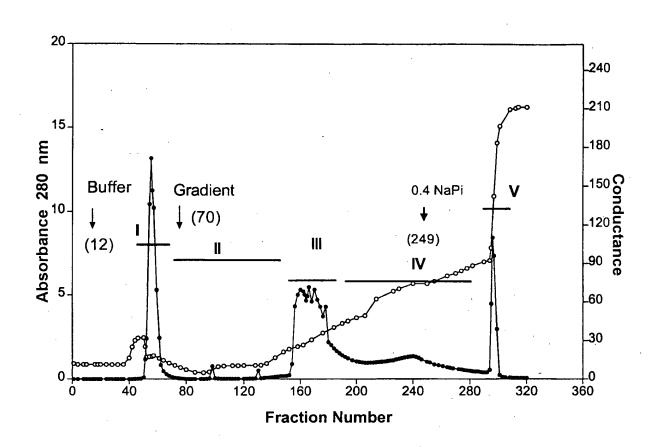
Contact: C.G. Mintz (713) 238-8000

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FIGURE 73

HTP BIO-GEL CHROMATOGRAPHY OF DEAE POOL IV



BARS = FRACTION POOLS

ARROWS = BUFFER CHANGES

LEGEND:

The Last that the transport of the Reservation

Open circles = Conductance

Closed circles = Absorbance

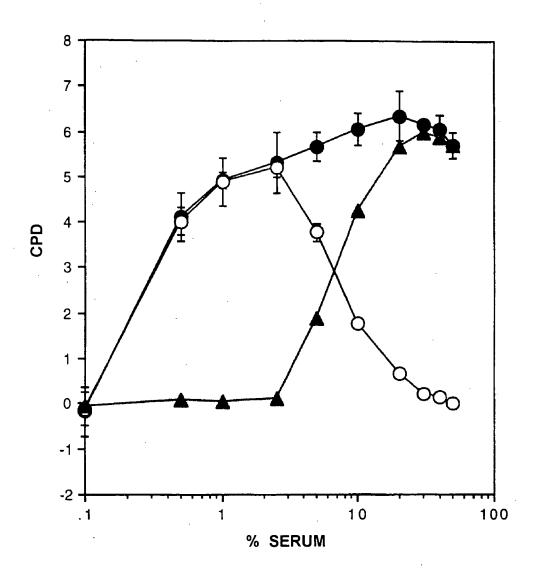
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 74

DIALYSIS OF CDE HORSE SERUM AGAINST TRIS BUFFER CONTAINING CALCIUM



LEGEND:

Open circles = $-E_2$

Closed circles = $+ E_2$

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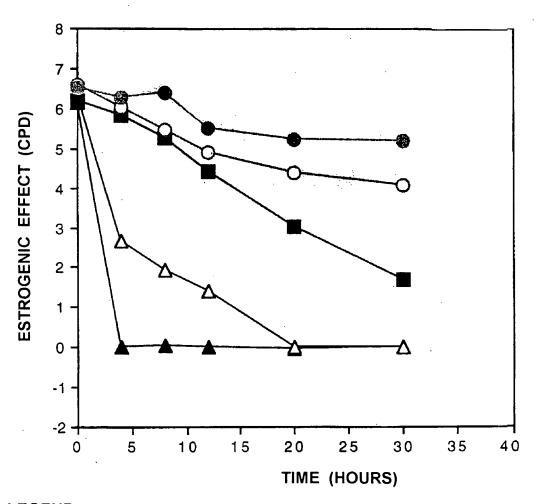
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THE EFFECT OF CALCIUM ON THE HEAT STABILITY OF THE INHIBITOR IN CDE HORSE SERUM (MTW9/PL2 CELLS)



LEGEND:

= Chelex treatment only

- = CDE horse serum

= Chelex and 1 mM calcium chloride

——— = Chelex and 10 mM calcium chloride

= Chelex and 50 mM calcium chloride

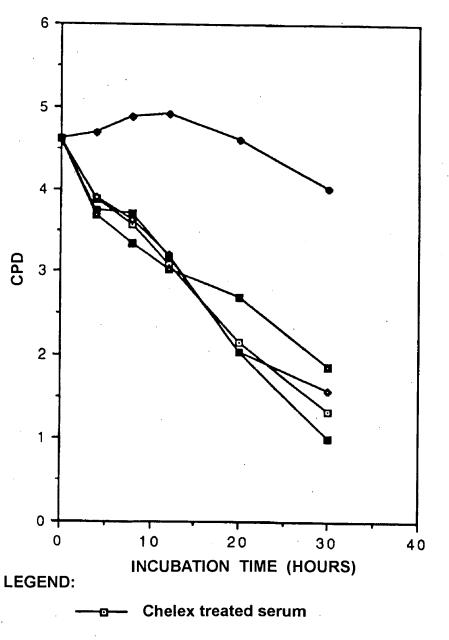
Inventor: Sirbasku

Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

Page 76 of 148 **FIGURE 76**

PROTECTIVE EFFECT OF METAL IONS ON CHELEX TREATED CDE HORSE SERUM INCUBATED AT 37° C AND ASSAYED WITH MTW9/PL2 CELLS



Chelex treated serum + 10 mM Calcium

Chelex treated serum + 50 uM Manganese

Chelex treated serum + 100 uM Magnesium

Chelex treated serum + 10 uM Zinc

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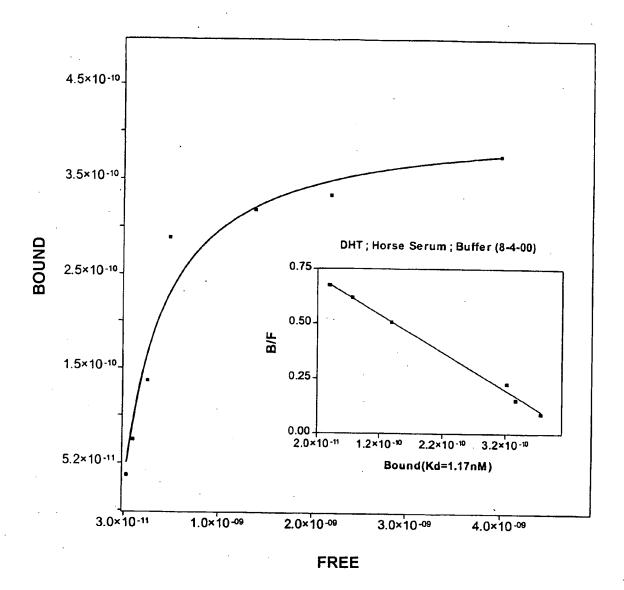
Express Mail EL81862334105 Inventor: Sirbasku

Contact: C.G. Mintz (713) 238-8000

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FIGURE 77

LABELED DHT BINDING TO CDE HORSE SERUM SATURATION ANALYSIS AND SCATCHARD PLOT



INSERT: Scatchard analysis of DHT binding

And the other wall the other than the start that

Inventor: Sirbasku

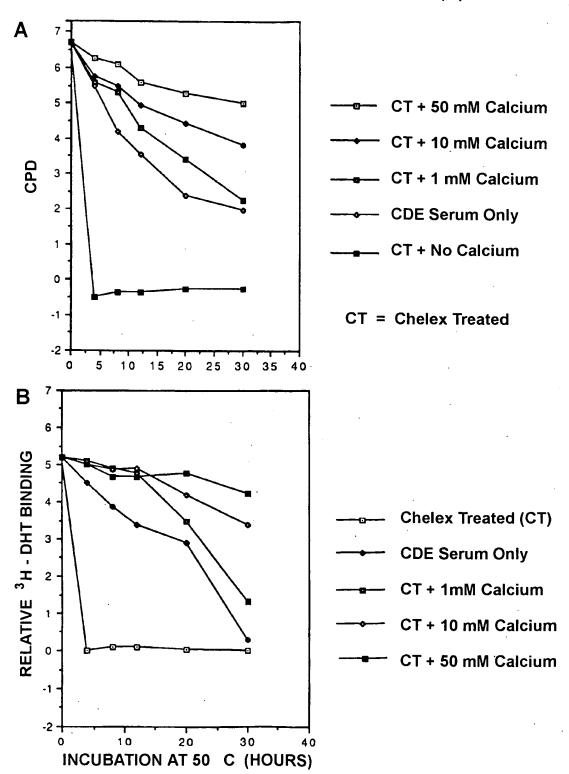
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EFFECT OF CALCIUM ON ESTROGENIC EFFECT (A) AND LABELED STEROID HORMONE BINDING (B)



Inventor: Sirbasku

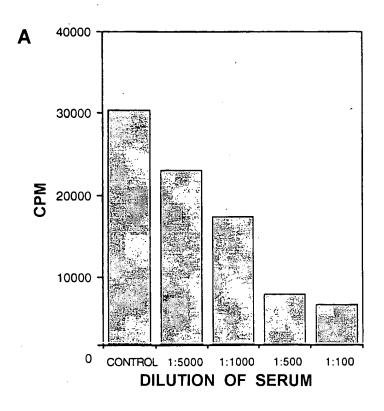
Atty Dkt. No. 1944-00201

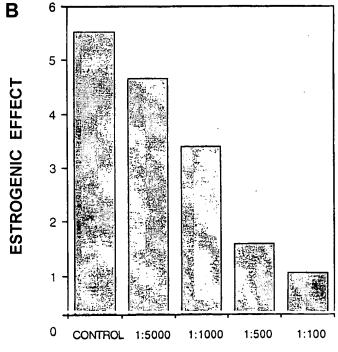
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FIGURE 79

ANTI - HUMAN SHBG PRECIPITATION OF THE LABELED DHT BINDING ACTIVITY (A) AND THE ESTROGENIC EFFECT IN CDE HORSE SERUM (B)





DILUTION OF ANTISERUM

Inventor: Sirbasku

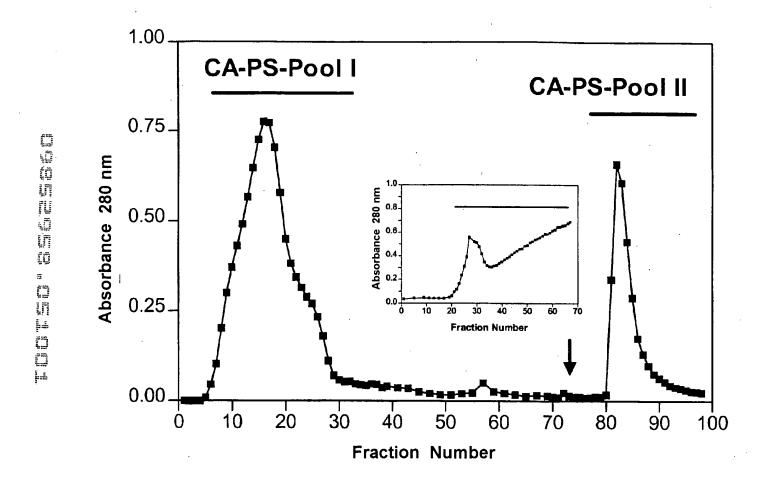
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FIGURE 80

PHENYL SEPHAROSE ELUTION OF CBG (CA-PS-POOL 1) AND SHBG-LIKE (CA-PS-POOL 11)



ARROW = ELUTION WITH 40% ETHYLENE GLYCOL

INSERT: CORTISOL AFFINITY COLUMN ELUTION

BARS = POOLED ACTIVE FRACTION

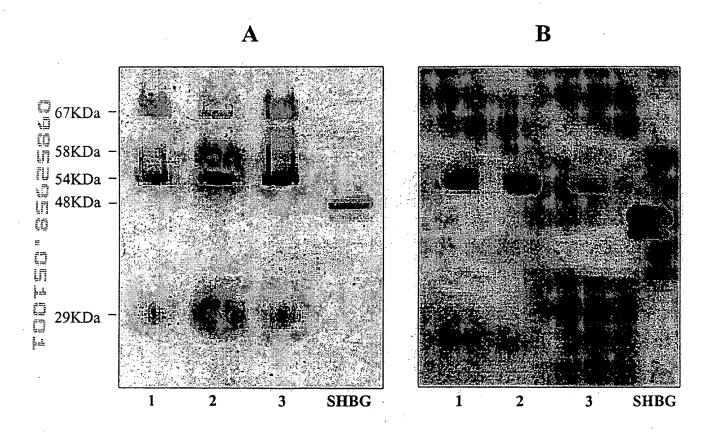
Express Mail EL818623541US Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 81

SDS PAGE (A) AND WESTERN ANALYSIS (B) OF THREE PREPARATIONS OF CA-PS-POOL II VS HUMAN SHBG



LANES 1, 2, AND 3 = 10 ug each of CA-PS-POOL II

LANE "SHBG" = 10 mg of purified protein

Inventor: Sirbasku

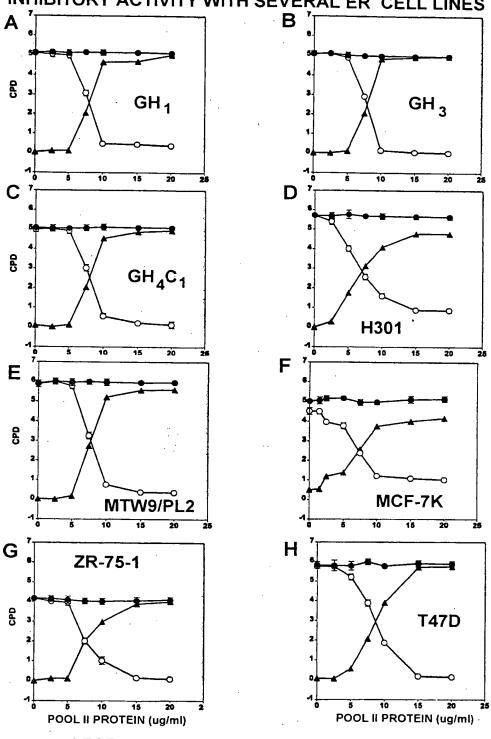
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FIGURE 82

ASSAY OF CA-PS-POOL II ESTROGEN REVERSIBLE INHIBITORY ACTIVITY WITH SEVERAL ER CELL LINES



LEGEND:

Open circles = $-E_2$

Closed circles = $+ E_2$

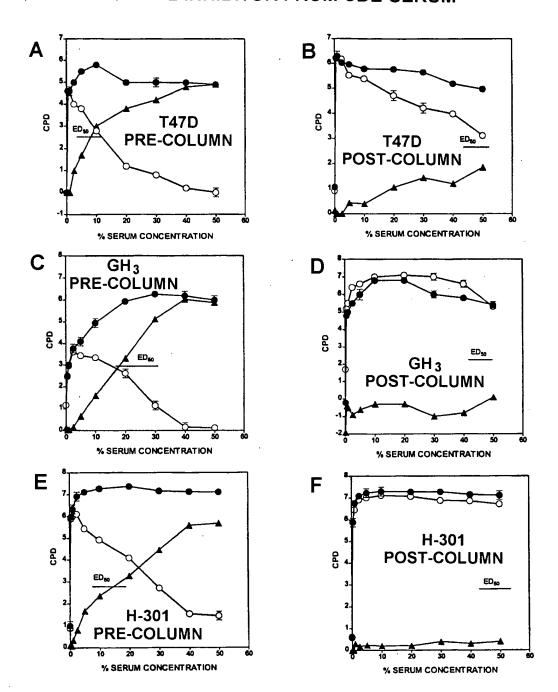
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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CORTISOL-AGAROSE AFFINITY REMOVAL OF THE INHIBITOR FROM CDE-SERUM



LEGEND:

Open circles $= - E_2$

Closed circles = $+ E_2$

Closed triangles = Estrogenic effect

H

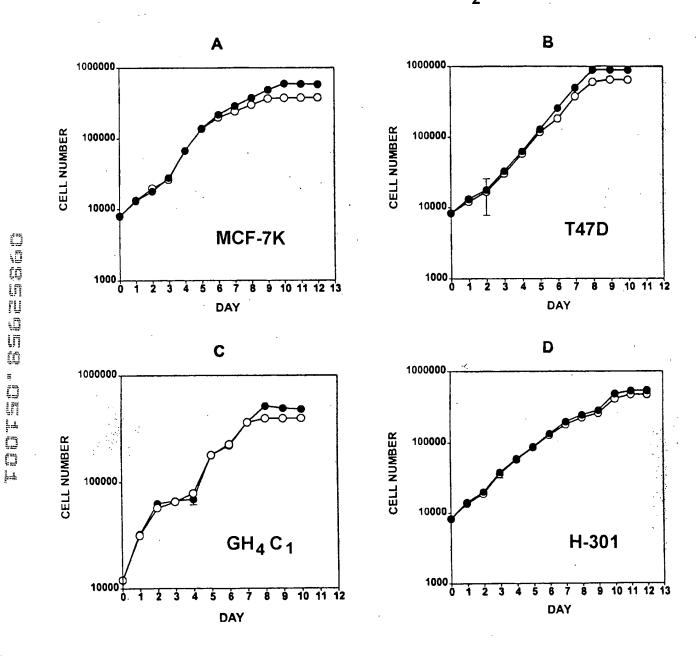
Inventor: Sirbasku

Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 84

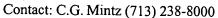
GROWTH OF ER+ CELL LINES IN SERUM-FREE MEDIUM ± E2



LEGEND:

Closed circles =
$$+ E_2$$

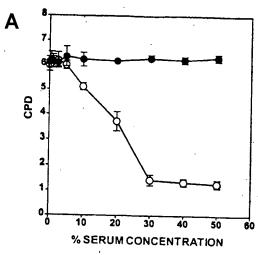
Open circles = $- E_2$



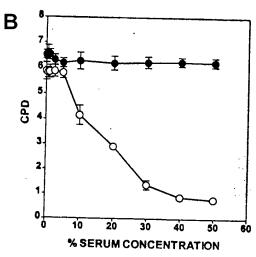
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EFFECT OF CDE-SERUM ON ESTROGEN RESPONSIVE GROWTH OF THREE ER + CANCER CELL LINES IN SFM



A = T47D IN DDM-2MF

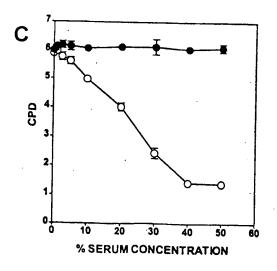


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To the that the tree

B = MTW9/PL2 IN DDM-2A



GH₄ C₁ IN PCM 9

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The state of the state of

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C

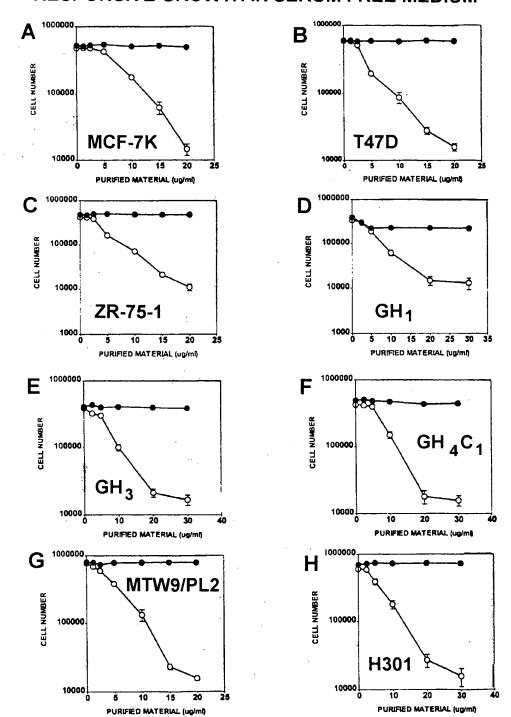
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

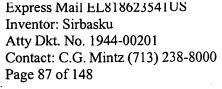
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EFFECT OF CA-PS-POOL II ON ESTROGEN RESPONSIVE GROWTH IN SERUM FREE MEDIUM



LEGEND: Open circles = $-E_2$ Closed circles = $+E_2$





AMINO ACID SEQUENCING - HORSE SHBG				
100 PRLDDGRW PRLDDGSW PRLNDGRW	200 CDVESNPGIFLPP CDVELQPGLFFPP CDVDLQPGLFFPQ * *** * #34:SVPGLVSPS	300 CMKALGLP CKKALGLP CMEVLSTS (TEVLGAS		
90 MAQLTVGAGI MAQLTVGAGI WARLTVGFGI WAQLTVGFGI	190 SLRSCDVESI SLRSCDVELG SLGNCDVDLG NLGNCDVDLG *	290 ISRVVLSQGSI STAVLSQGSI FKVALSQGPI LKVVLSQGPP		
80 GRPE I QLHNH GRPE I QLHNI GQLE I QMHNF	180 SWLDPQAGESASAPT SWLDPQAGESASAHL IWLGHQAGESTSART TWLGHQVHESPSAP. *** F #20:VQESPse	280 SLPLQLKLSM SLPLQLKLGV SLPLQLKLDI		
70 DDWFMLGLRD DDWFMLGLRD DDWFMLGLRD	160 170 180 PLVPALDGCLRRDSWLDKQAEISASA PLVPALDGCIRRDIWLGHQAQLSTSA PLVPALDGCIRRDTWLGHQAQLSTSA PLVPALDGCIRRDTWLGHQVHLSPSA * * * * * * * * * * * * * * * * * * *	270 GPGLDLPLVL EPGLDLPLAW EPKLALPLAW EPKLVLALDM **** VPK #9:LAVQVR	370 SPGNGTDASH SPGNGTDASH SPSNDTHTSH SPNNVSHISH	
60 /IFYGDTNPKI /IFYGDTNPEI /IFYGDTNTEI /IFYGDTNTKI **	160 LPLVPALDGG LPLVPALDGG EPLVPALDGG FPLVPALDGG #37:ATVV	260 QDQKVVLSSGSGPG QDQKVVLSSGMEPG QDQTVVLSSEAEPK QDQKVLSSGVEPK ** ** #26:VVVILAIVPK	360 EIWTHSCPQS IDIWTHSCPQS IDIWTHSCPQS	
50 60 EVRTWDPEGVIFYGDT ELRTWDPEGVIFYGDT EFRTLDPEGVIFYGDT ****	150 LGGLLFPPSSLI VGGLLFPPSSLI LGGLLLPTSKLI LGGLLLPTSSLI LGGLLPTSSLI VPGLVSPSQ	250 PSWLSLHLQI PNWLSLHLQI SSWLTLHLQI SSWLSLHLQI SSWLSLHLQI	350 DVDQALNRSH DMDKALNRSH DIDKALSRSG DIDQALNRSG	
10 20 30 90 100 TQSAHDPPAVHLSNGPGQEPIAVMTFDLTKITKTSSSFEVRTWDPEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNHWAQLTVGAGPRLDDGRW TQRAQDSPAVHLINGLGQEPIQVLTFDLTRIVKASSSFELRTWDSEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNLWAQLTVGAGPRLDDGSW IQSAQDSPAKYLSNGPGQEPVTVLTIDLTKISKPSSSFEFRTWDPEGVIFYGDTNTEDDWFMLGLRDGQLEIQLHNLWARLTVGFGPRLNDGRW *** * * * * * * * * * * * * * * * * *	130 140 150 160 170 180 200 SQVSGPLTSKRHPIMRIALGGLLFPASNIRLPLVPALDGCLRRDSWLDKQAEISASAPTSLRSCDVESNPGIFLPP SQVSGTLHDKPQPVMKIAVGGLLFPPSSLRLPLVPALDGCLRRGSWLDPQAQLSASAHLSLRSCDVELQPGLFFPP RQVSASLADHPQLSMRIALGGLLPTSKLRFPLVPALDGCIRRDIWLGHQAQLSTSARTSLGNCDVDLQPGLFFPP RQISGTLANNSWPSMRIALGGLLLPTSSLRFPLVPALDGCLRRDTWLGHQVHLSPSAP.NLGNCDVDLQPGLFFPP * ** * * * * * * * * * * * * * * * *	230 240 260 300 280 300 300 SGELKLSMSRVVLSQGSKMKALALP SELKERSGRENTLALGDQKVVLSSGSGPGLDLPLVLGLPLQLKLSMSRVVLSQGSKMKALALP SELKPSEGSGRLLALGTPEDPNWLSLHLQDQKVVLSSGMEPGLDLPLAWGLPLQLKLGVSTAVLSQGSKKKALGLPLGFKLVDGAGRLLTLGTGTNSSWLTLHLQDQKVVLSSEAEPKLALPLAVGLPLQLKLDVFKVALSQGPKMEVLSTS KKLVDGSGCLLALGTRTNSSWLSLHLQDQKVVLSSGVEPKLVLALDMGLPLQLKLDILKVVLSQGPKTEVLGAS **** ##26:VVVILAIVPK #9:LAVQVR	330 340 350 350 350 350 350 370 3EDSSTSFCLNGLWAQGQRLDVDQALNRSHEIWTHSCPQSPGNGTDASH 3EDSSASFCLDGLWTVGQKLDMDKALNRSHDIWTHSCPQSPGNGTDASH 3EDSSASFCLSDLWVQGQRLDIDKALSRSQDIWTHSCPQSPSNDTHTSH 3DNSSASFCLSDLWVQGQRLDIDQALNRSQNIWTHSCPHSPNNVSHISH	
30 EPIAVMTFDL EPIGVLTFDL EPVAVMTIDL * ** *	130 RQVSGPLTSKR SQVSGTLHDKP RQVSASLADHP RQISGTLANNS * * *	230 LGLKQAAGSG LELKPSEGSG LGFKLVDGAG LGLKLVDGSG	330 GEDSSTSFCL GEDSSASFCL GEDSSASFCL GDNSSASFCL	
10 20 SAHDPPAVHLSNGPGG RAQDSPAVHLINGLGG SAQDSPAKYLSNGPGGNGPGG	120 EVDGEEVLRLI EVDGKENLCLI WYDGKELLCLI WYDGKELLCLI	220 HAEPWAFSLD QTEPWAFSLD HTDPWSFSLE RTDPWSFSLE	320 QGRLFLGALP QGRLFLGALP QGRLSLGALP QGLLSLGALA	
10 20 30 40 50 60 70 80 90 100 LRPVLPTQSAHDPPAVHLSNGPGQEPIAVMTFDLTKITKTSSSFEVRTWDPEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNHWAQLTVGAGPRLDDGRW TQRAQDSPAVHLINGLGQEPIQVLTFDLTRIVKASSSFELRTWDSEGVIFYGDTNPKDDWFMLGLRDGRPEIQLHNLWAQLTVGAGPRLDDGSW LRHIDPIQSAQDSPAKYLSNGPGQEPVTVLTIDLTKISKPSSSFEFRTWDPEGVIFYGDTNTEDDWFMLGLRDGQLEIQLHNLWARLTVGFGPRLNDGRW * * * * * * * * * * * * * * * * * * *	110 120 130 140 150 160 170 170 180 200 200 190 EQUEVENCENCENCENCENCENCENCENCENCENCENCENCENCE	210 220 GTQAEFNLRDIPQPHAEPWAFSLDI GTHAEFSLQDIPQPHTDPWTFSLDI GTHAEFSLQDIPQPRTDPWSFSLEI K * **: Q #41:VFALAPIPGVLK	310 320 340 340 350 370 SPIGLFLGALPGEDSSTSFCLNGLWAGGGRLDVDGALNRSHEIWTHSCPGSPGNGTDASH SPGLGPLNLWAKPQGRLFLGALPGEDSSTSFCLNGLWTVGGKLDMDKALNRSHEIWTHSCPGSPGNGTDASH LERLASLWRLWSHPQGRLSLGALPGEDSSASFCLSDLWYGGGRLDIDKALSRSQDIWTHSCPQSPSNDTHTSH ASRLAALRTLWSHPQGRLSLGALPGEDSSASFCLSDLWYQGGRLDIDKALSRSQDIWTHSCPHSPNNVSHISH	
hm SHBG Irb SHBG rt ABP I	hm SHBG Frb SHBG Frt ABP Frb ABP Frb	hm SHBG Crb SHBG Crt ABP Crb A	hm SHBG I rb SHBG S rt ABP I hs ABP	

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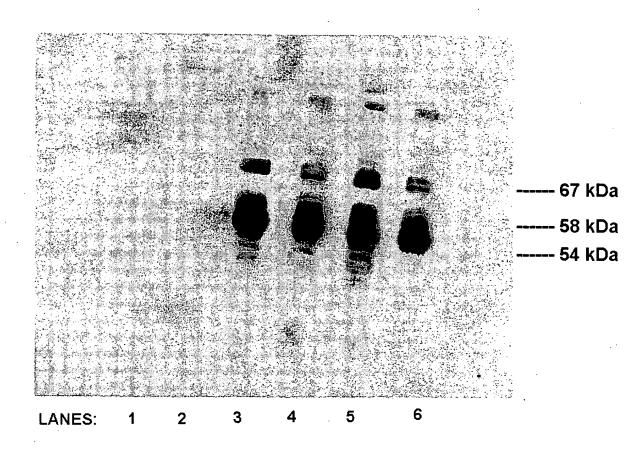
Contact: C.G. Mintz (713) 238-8000

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FIGURE 88

WESTERN ANALYSIS OF CBG (POOL I) AND

SHBG (POOL II) PREPARATION WITH ANTI-54 kDa REST AVAILABLE COPY



1 = CBG PREPARATION #5

2 = CBG PREPARATION #6

3 = SHBG PREPARATION #5.1

4 = SHBG PREPARATION #5.2

5 = SHBG PREPARATION #6.1

6 = SHBG PREPARATION #6.2

ANTIBODY = RABBIT ANTI-54 kDa 1:5000 DILUTION

The control of the co

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Inventor: Sirbasku

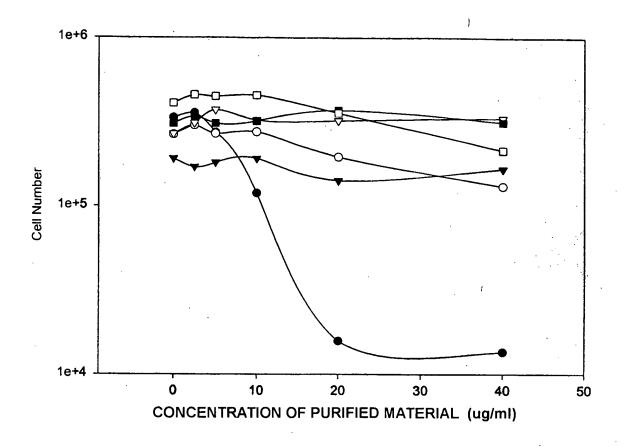
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EFFECT OF ANTI-54kDa ANTISERUM ON MTW9/PL2 CELLS GROWN IN THE PRESENCE OF CA-PS-POOL II



LEGEND:

● No antibody

--∕- Antibody 1:5000

→ Antibody 1:1000

--**▽** Antibody 1:500

Antibody 1:100

--□- Antibody 1:50

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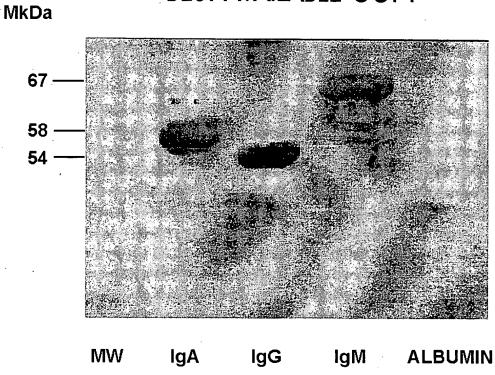
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FIGURE 90

WESTERN BLOT OF COMMERCIAL PREPARATIONS OF HORSE IgA, IgG AND IgM WITH THE ANTI-54 kDa ANTIBODY

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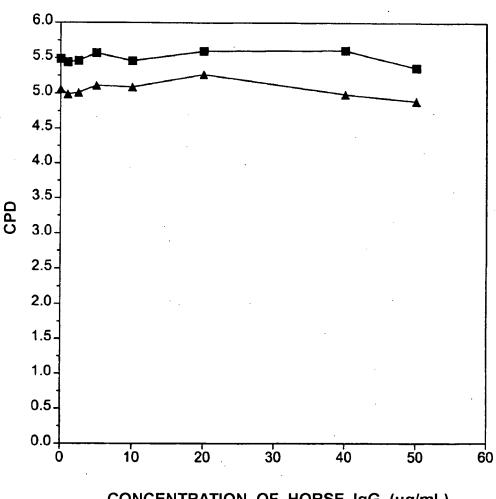
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I 148

FIGURE 91

EFFECT OF COMMERCIALLY PURIFIED HORSE IgG ON MTW9/PL2 CELL GROWTH IN 2.5% CDE-HORSE SERUM



CONCENTRATION OF HORSE IgG (ug/mL)

LEGEND: — plus E₂

—— minus E₂

Inventor: Sirbasku

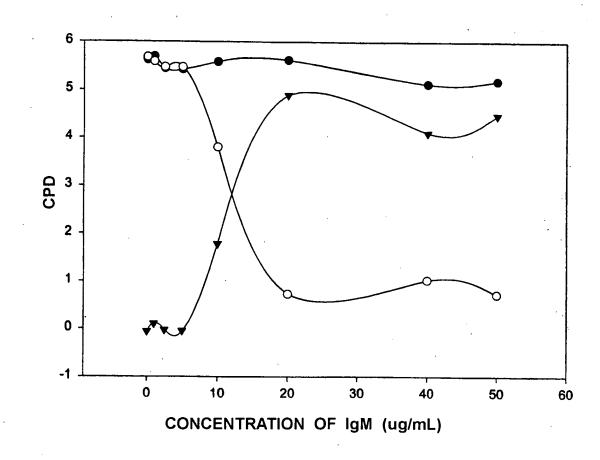
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EFFECT OF HORSE IgM ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM \pm E $_2$



LEGEND:

In the fact the way that a

= Estrogenic effect

Inventor: Sirbasku

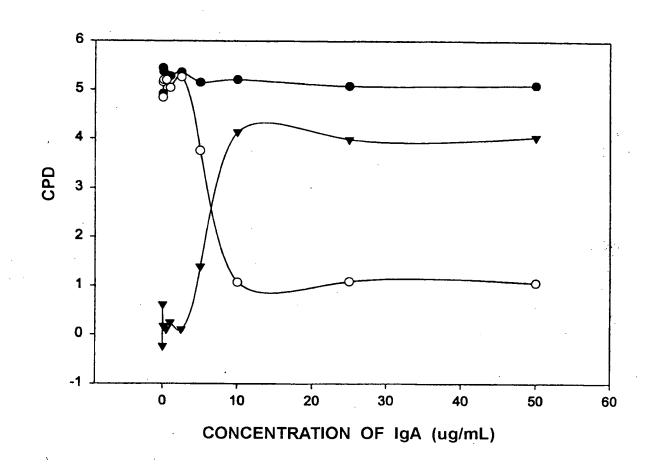
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EFFECT OF HORSE IgA ON GROWTH OF THE MTW9/PL2 CELLS IN 2.5% CDE HORSE SERUM \pm E $_2$



LEGEND:

The first that the first

Ü

→ = Estrogenic effect

Inventor: Sirbasku

Atty Dkt. No. 1944-00201

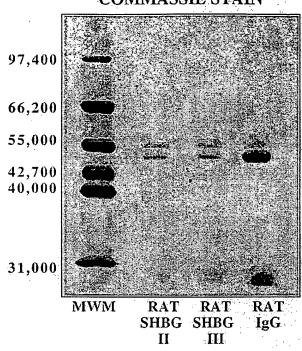
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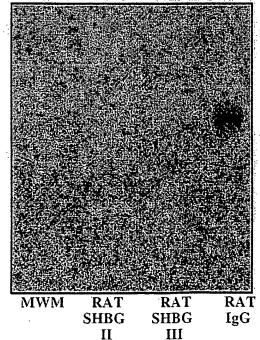
FIGURE 94

SDS PAGE AND WESTERN ANALYSIS OF RAT "SHBG-LIKE" PREPARATIONS

COMMASSIE STAIN



WESTERN BLOT. ANTI IgG



SDS PAGE

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WESTERN ANALYSIS WITH ANTI-RAT IgG Express Mail EL818623541US Inventor: Sirbasku

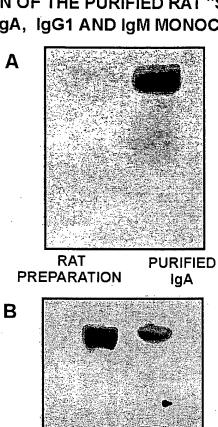
Atty Dkt. No. 1944-00201

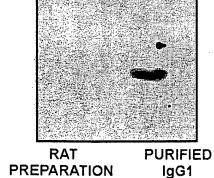
Contact: C.G. Mintz (713) 238-8000

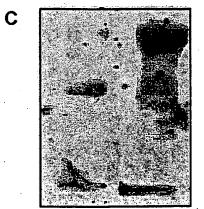
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CROSSREACTION OF THE PURIFIED RAT "SHBG-LIKE" PROTEINS WITH ANTI- IgA, IgG1 AND IgM MONOCLONAL ANTIBODIES







RAT PREPARATION

PURIFIED IgM

Inventor: Sirbasku
Atty Dkt. No. 1944-00201
Contact: C.G. Mintz (713) 238-8000
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FIGURE 96

AMINO ACID SEQUENCING - RAT SHBG

rbSHBG rbSHBG hsABP rtABP	10 20 30 40 50 60 70 80 90 100
hmSHBG rbSHBG hsABP rtABP	110 120 130 140 150 160 170 180 200 200Q. V. E. V. E. E. V. R GP. TSKRHPI F. A. N. L L S DK EI. A P RS ESN I. L
hmSHBG rbSHBG hsABP rtABP	#18:vvsGLFPvP 210
hmSHBG rbSHBG hsABP rtABP	310 320 1 350 350 370 P.G. P.LN. AK F

Inventor: Sirbasku

Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

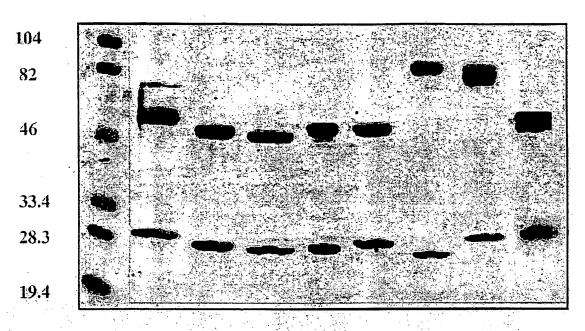
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FIGURE 97

SDS PAGE (A) AND WESTERN ANALYSIS (B) WITH ANTI-SHBG AND RAT Ig'S

A KDa RAT I

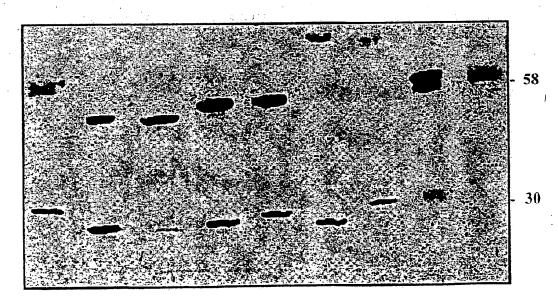
RAT Igs COMMASSIE STAINED



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MW IgA IgG1 IgG2a IgG2b IgG2c IgE IgM RP

B RAT Igs WESTERN BLOT. ANTI SHBG ANTIBODY KDa



lgA IgG1 IgG2a IgG2b IgG2c IgE IgM HP RP

REST AVAILABLE COPY

Inventor: Sirbasku

Atty Dkt. No. 1944-00201

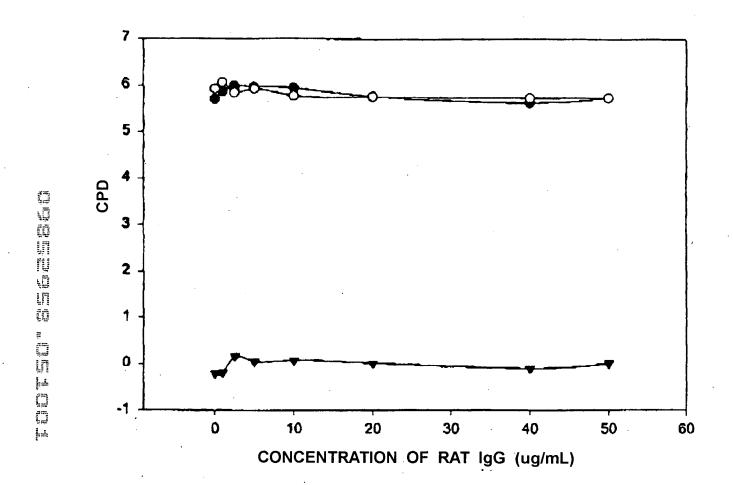
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FIGURE 98

EFFECT OF RAT IgG ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Inventor: Sirbasku

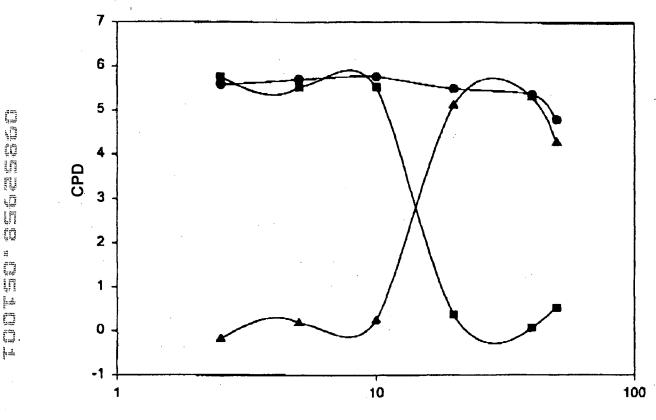
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FIGURE 99

GROWTH IN 2.5% CDE RAT SERUM



CONCENTRATION OF RAT IgA (ug/mL)

LEGEND:

Closed circles $= + E_2$

Closed squares = $-E_2$

Inventor: Sirbasku

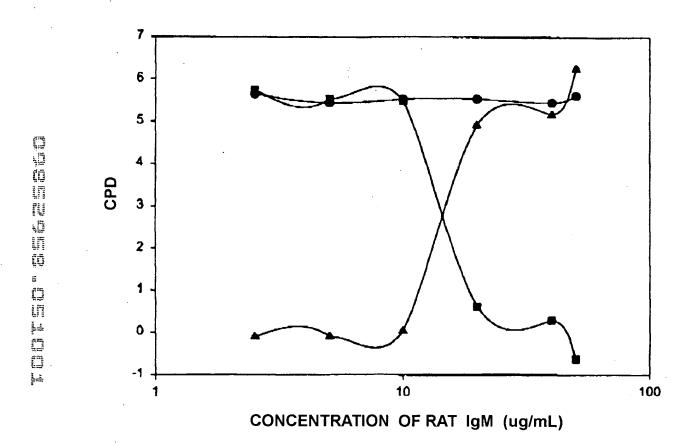
Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 100

EFFECT OF RAT IgM ON MTW9/PL2 CELL GROWTH IN 2.5% CDE RAT SERUM



LEGEND:

Closed squares = $-E_2$

Closed circles = + E₂

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Inventor: Sirbasku

Atty Dkt. No. 1944-00201

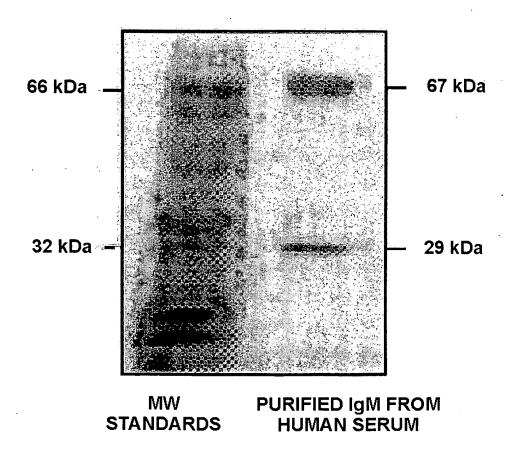
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ELUTION OF IGM FROM MANNAN BINDING PROTEIN COLUMN

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Express Mail EL818623541US Inventor: Sirbasku

Atty Dkt. No. 1944-00201

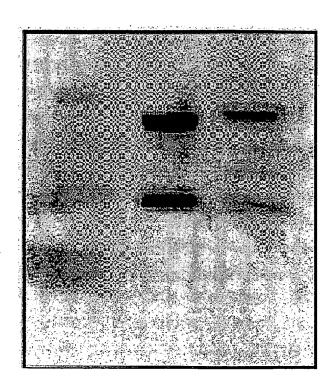
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FIGURE 102

Igm Purification From Plasma by Jacalin

BEST AVAILABLE COPY



MW HUMAN PURIFIED IgA IgA

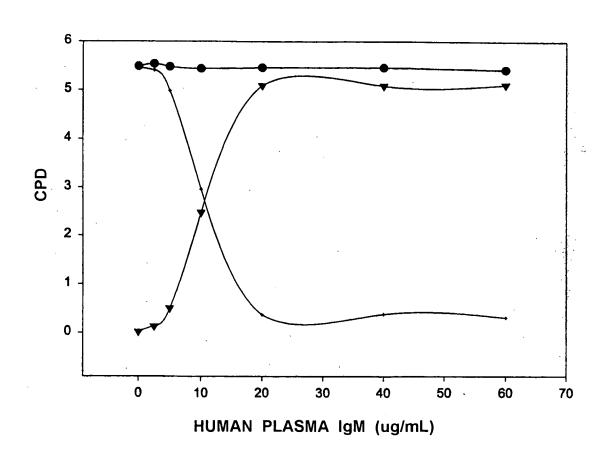
Inventor: Sirbasku

Atty Dkt. No. 1944-00201 Contact: C.G. Mintz (713) 238-8000

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FIGURE 103

EFFECT OF IgM ISOLATED FROM HUMAN PLASMA ON MTW9/PL2 GROWTH IN SERUM-FREE CONDITIONS



LEGEND:

Ü

= Estrogenic effect

Inventor: Sirbasku

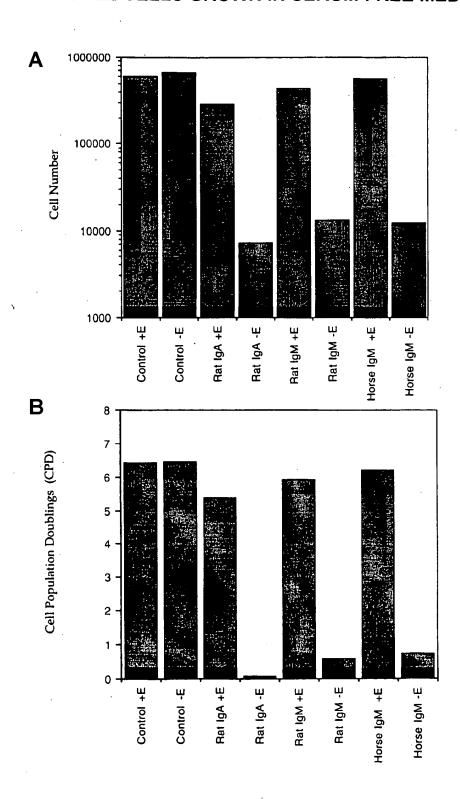
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THE EFFECT OF VARIOUS IGA AND IGM PREPARATIONS ON MTW9/PL2 CELLS GROWN IN SERUM-FREE MEDIUM



Languess Mail EL818623541US Inventor: Sirbasku

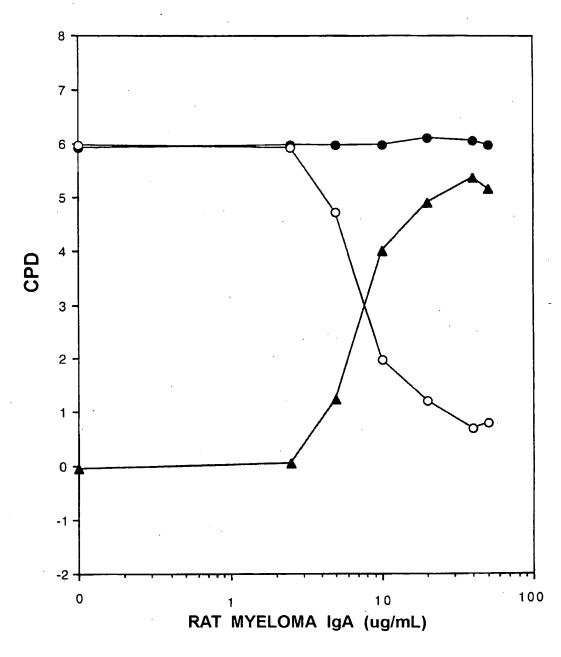
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FIGURE 105

RAT MYELOMA IGA TITRATION ON GH₁ CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

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Express Mail EL818623541US

Inventor: Sirbasku

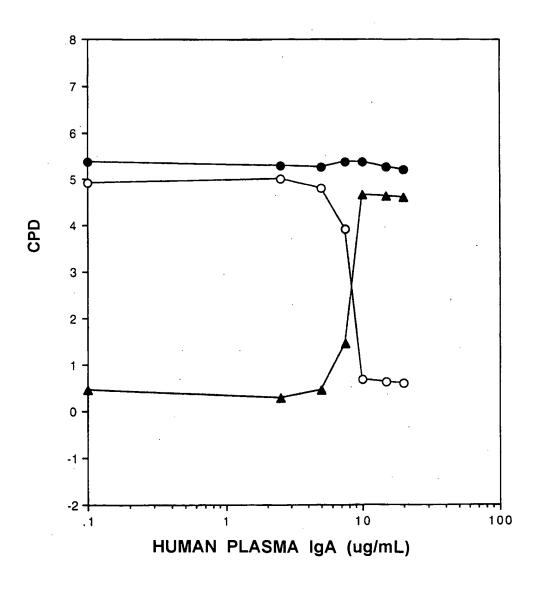
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Contact: C.G. Mintz (713) 238-8000

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FIGURE 106

HUMAN PLASMA IGA TITRATION ON GH₁ CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Inventor: Sirbasku

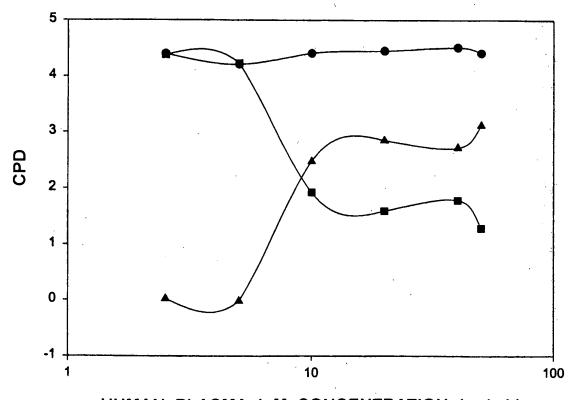
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FIGURE 107

HUMAN PLASMA IgM TITRATION ON GH₁ CELLS GROWN IN SERUM-FREE CONDITIONS



HUMAN PLASMA IgM CONCENTRATION (ug/mL)

LEGEND:

The true and the Las had

= Estrogenic effect

Inventor: Sirbasku

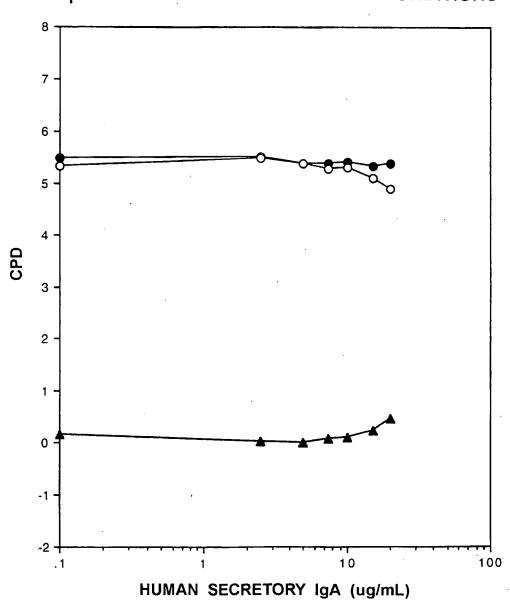
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Contact: C.G. Mintz (713) 238-8000

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FIGURE 108

EFFECT OF HUMAN SECRETORY IGA ON GH₁ CELLS GROWN IN SERUM-FREE CONDITIONS



LEGEND:

Closed circles = + E₂

Open circles = $-E_2$



Inventor: Sirbasku

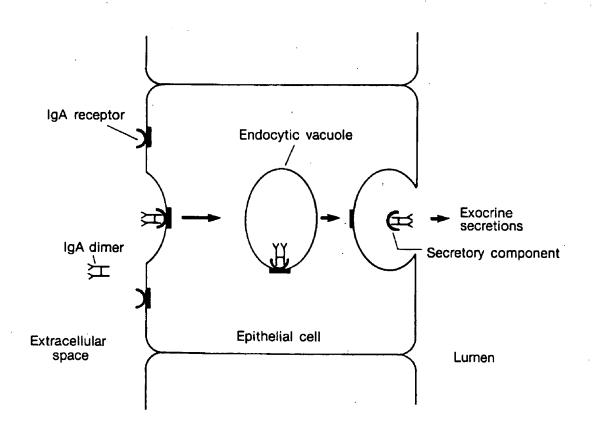
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Contact: C.G. Mintz (713) 238-8000

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FIGURE 109

MECHANISM OF TRANSCYTOSIS OF IgA AND IgM BY MUCOSAL EPITHELIAL CELLS



Inventor: Sirbasku

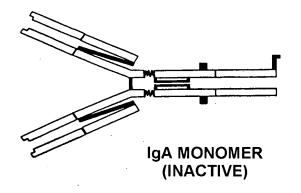
Atty Dkt. No. 1944-00201

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FIGURE 110

ESSENTIAL STRUCTURES OF HUMAN PLASMA AND SECRETORY IGA





J CHAIN





IgA DIMER WITH ATTACHED J CHAIN (ACTIVE)

SECRETORY PIECE OR SECRETORY COMPONENT (80% POLY-IgR)



SECRETORY IGA SHOWING J CHAIN AND SECRETORY COMPONENT (INACTIVE)

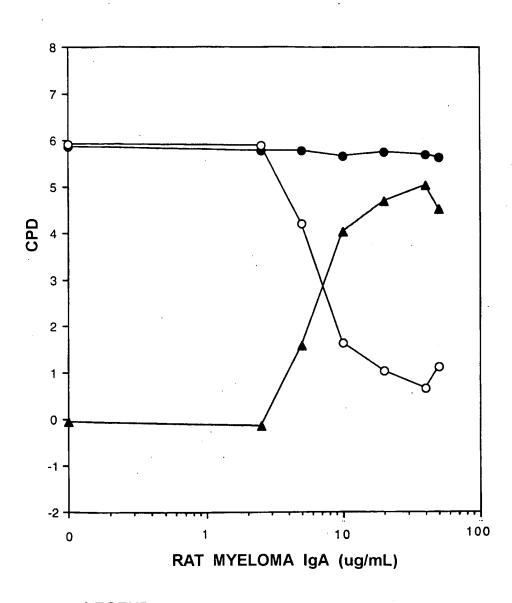
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 111

EFFECT OF RAT MYELOMA IGA ON GH 3 CELLS GROWN IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = + E₂

Open circles = $-E_2$

Closed triangles = Estrogenic effect

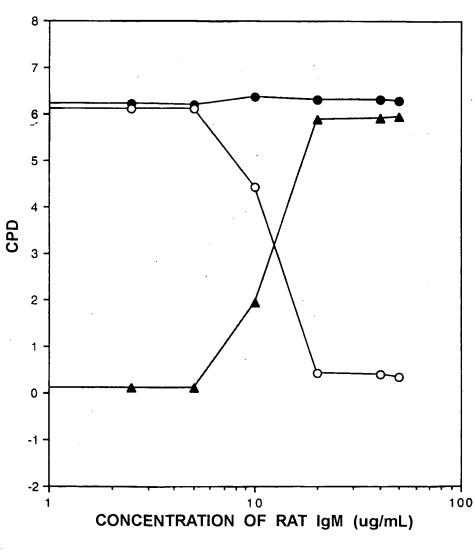
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 112

EFFECT OF RAT IgM ON GH₃ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

→ = Estrogenic effect

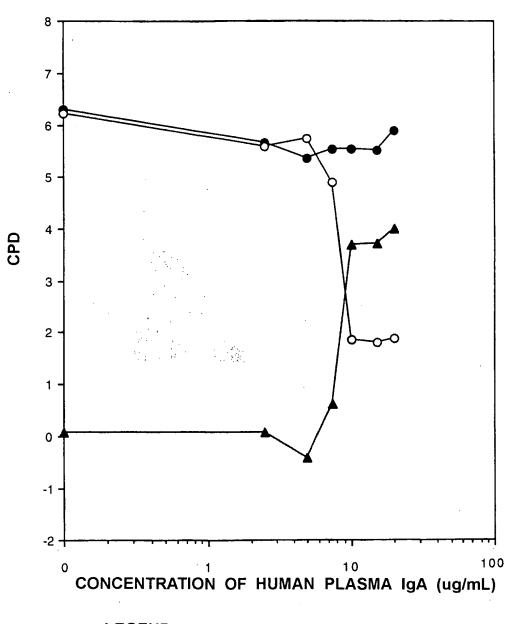
 Inventor: Sirbasku

Atty Dkt. No. 1944-00201 Contact: C.G. Mintz (713) 238-8000

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FIGURE 113

EFFECT OF HUMAN PLASMA IGA ON GH₃ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

 Inventor: Sirbasku

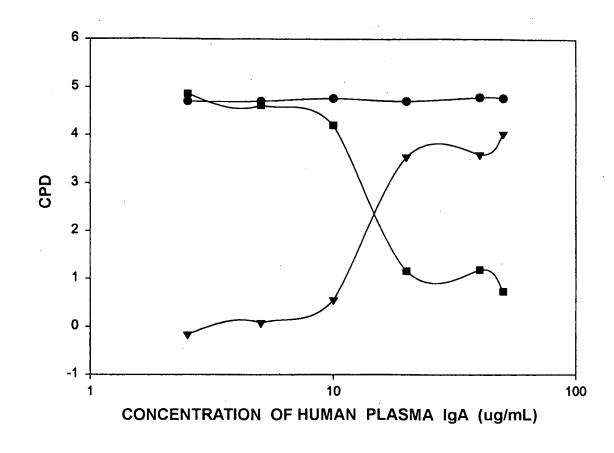
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Contact: C.G. Mintz (713) 238-8000

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EFFECT OF HUMAN PLASMA IgM ON GH $_{\rm 3}$ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

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→ = Estrogenic effect

Inventor: Sirbasku

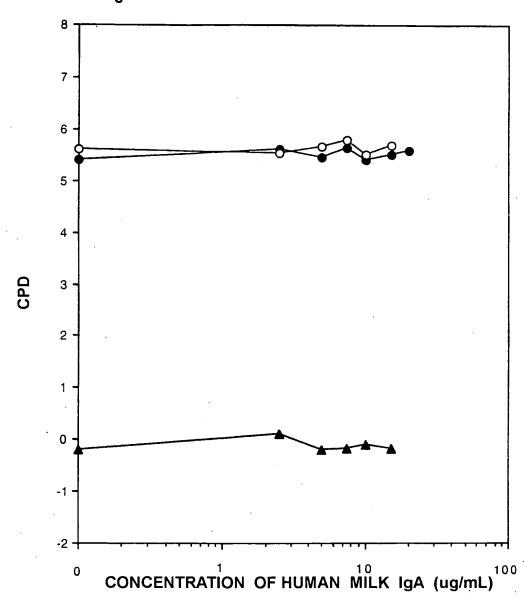
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FIGURE 115

EFFECT OF HUMAN MILK SECRETORY IGA ON GH₃ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

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Inventor: Sirbasku

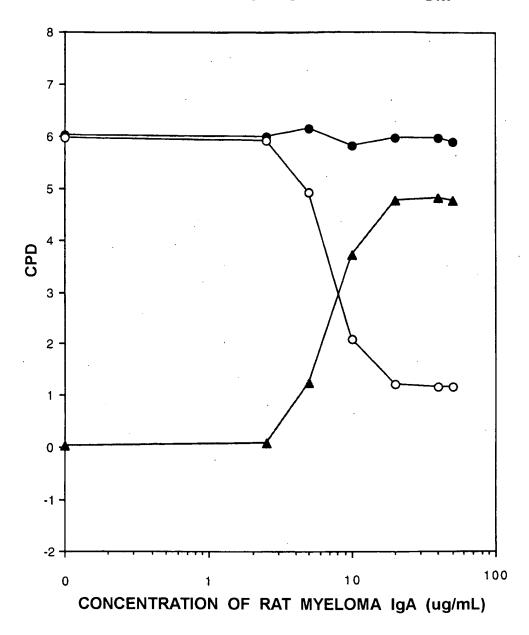
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FIGURE 116

EFFECT OF RAT MYELOMA IgA ON GH₄ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles $= -E_2$

Closed triangles = Estrogenic effect

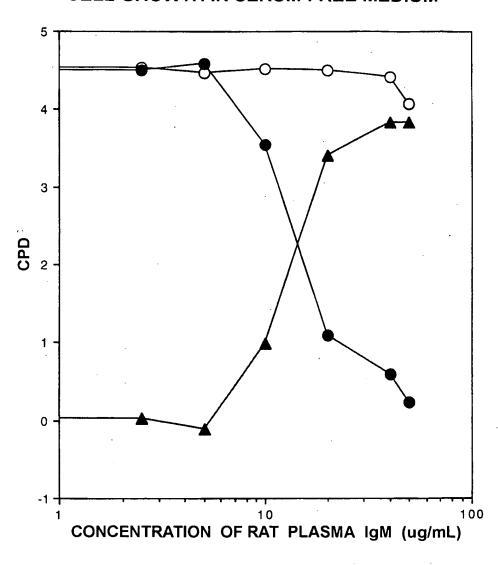
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 117

EFFECT OF RAT PLASMA IgM ON GH₄ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

→ = Estrogenic effect

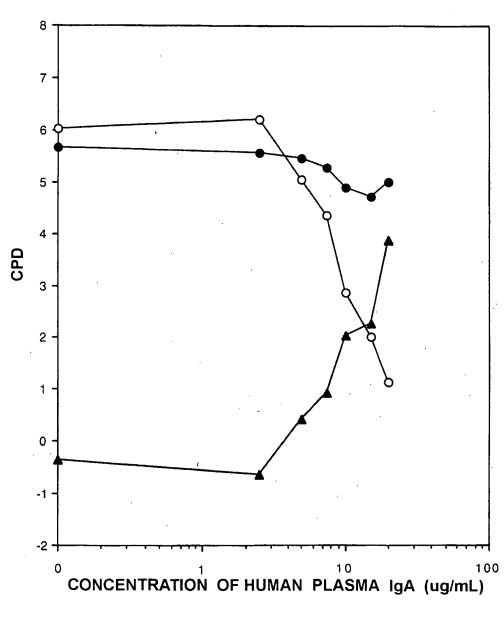
Inventor: Sirbasku Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 118

EFFECT OF HUMAN PLASMA IGA ON GH₄C₁ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

Inventor: Sirbasku

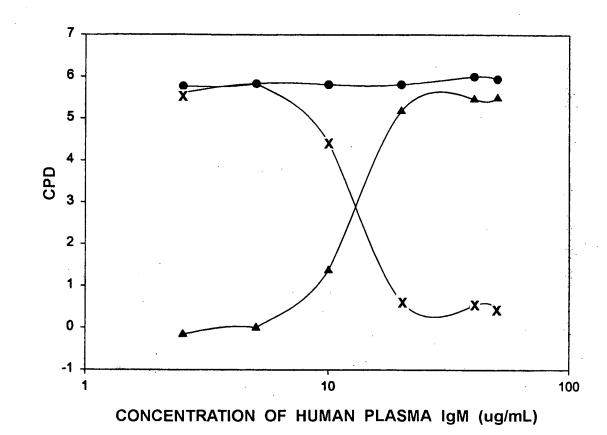
Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 119

EFFECT OF HUMAN PLASMA IGM ON GH₄C₁ CELL GROWTH IN SERUM-FREE MEDIUM



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Inventor: Sirbasku

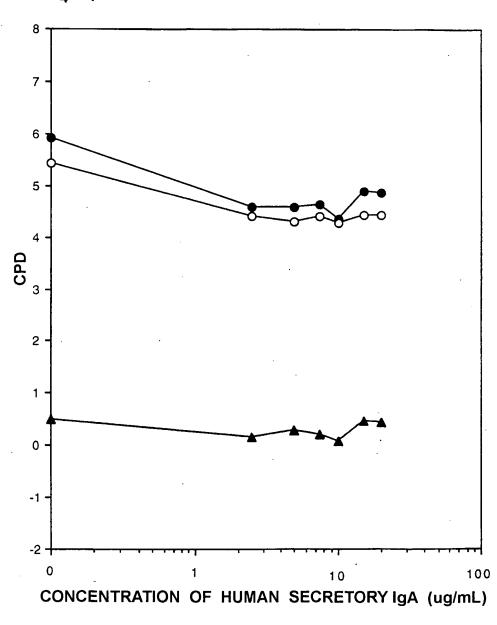
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FIGURE 120

EFFECT OF HUMAN MILK SECRETORY IGA ON GH₄C₁ CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

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Closed circles = $+ E_2$

Open circles $= - E_2$

Closed triangles = Estrogenic effect

Inventor: Sirbasku

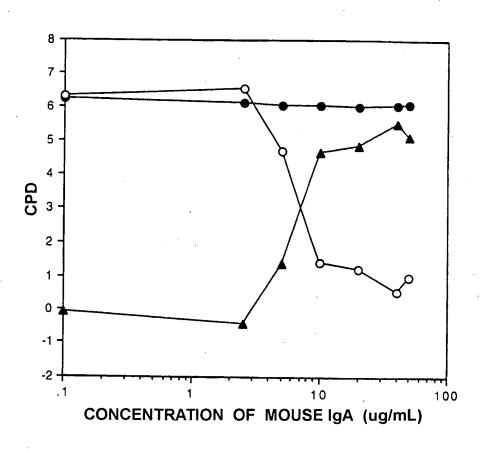
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FIGURE 121

EFFECT OF MOUSE IgA ON H301 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

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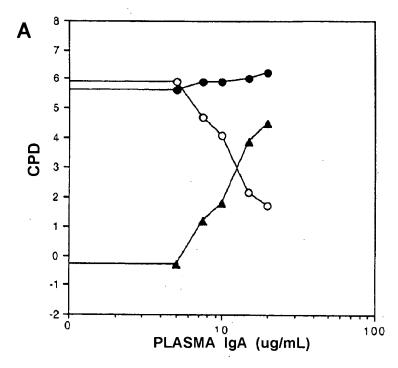
Inventor: Sirbasku Atty Dkt. No. 1944-00201

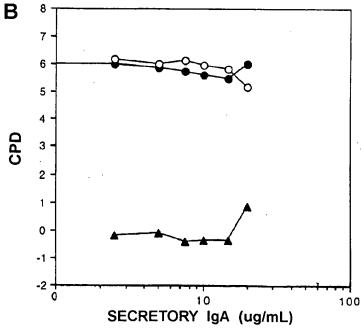
Contact: C.G. Mintz (713) 238-8000

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FIGURE 122

EFFECT OF HUMAN PLASMA IGA (A) AND SECRETORY IGA (B) ON H301CELL GROWTH IN SERUM-FREE MEDIUM





LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

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Inventor: Sirbasku

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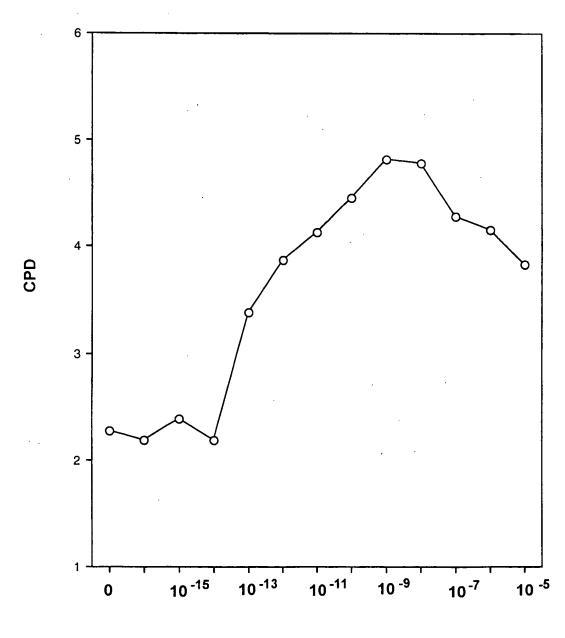
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FIGURE 123

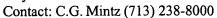
EFFECT OF ESTRADIOL ON H301 CELL GROWTH IN SERUM-FREE MEDIUM AND 40 ug/mL OF HUMAN IgM



ESTRADIOL CONCENTRATION (M)

Inventor: Sirbasku

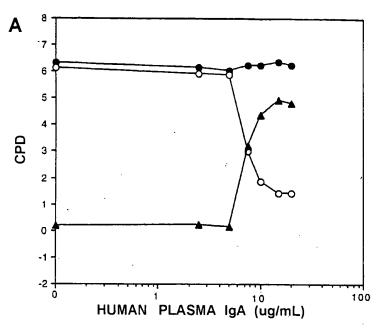
Atty Dkt. No. 1944-00201

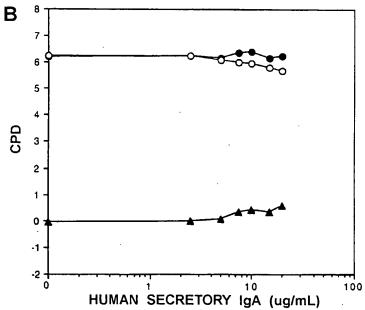


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FIGURE 124

EFFECT OF HUMAN PLASMA IGA (A) AND SECRETORY IGA (B) ON MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM



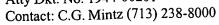


LEGEND: Close

Closed circles = $+ E_2$

Open circles = - E 2

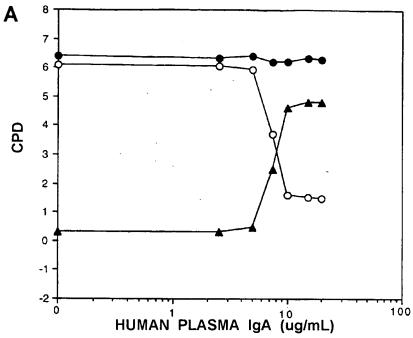
Closed triangles = Estrogenic effect

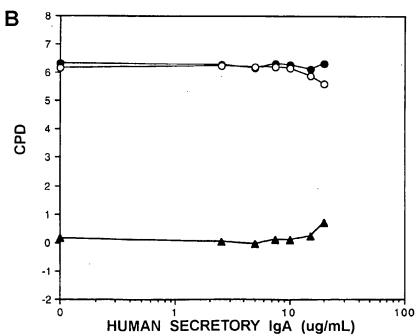


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FIGURE 125

EFFECT OF HUMAN PLASMA IGA (A) AND SECRETORY IGA (B) ON MCF-7KCELL GROWTH IN SERUM-FREE MEDIUM





LEGEND: Closed cir

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

Inventor: Sirbasku

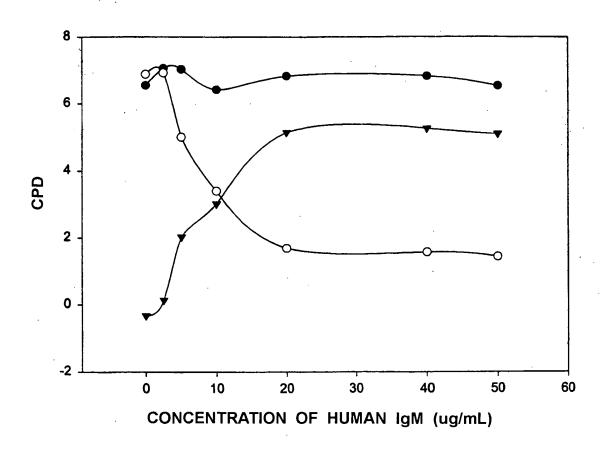
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Contact: C.G. Mintz (713) 238-8000

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FIGURE 126

EFFECT OF HUMAN IgM ON MCF-7A CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

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Inventor: Sirbasku

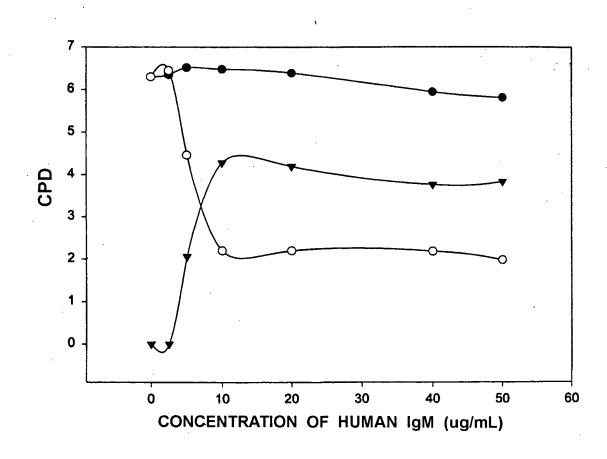
Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 127

EFFECT OF HUMAN IGM ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM



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express Maii elői 862334108 Inventor: Sirbasku

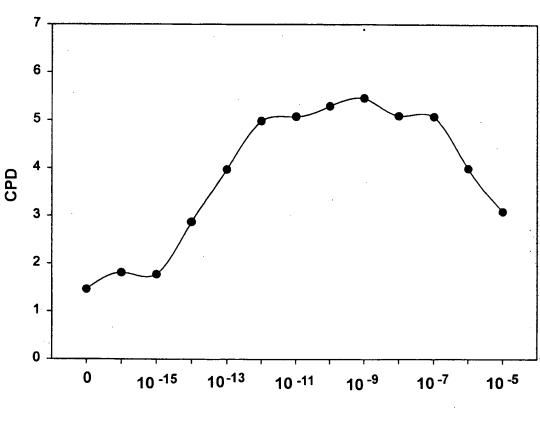
Atty Dkt. No. 1944-00201

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EFFECT OF ESTRADIOL ON MCF-7K CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM



CONCENTRATION OF ESTRADIOL (M)

Inventor: Sirbasku

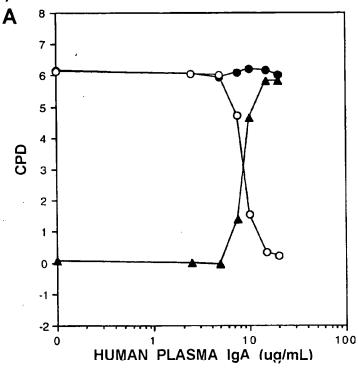
Atty Dkt. No. 1944-00201

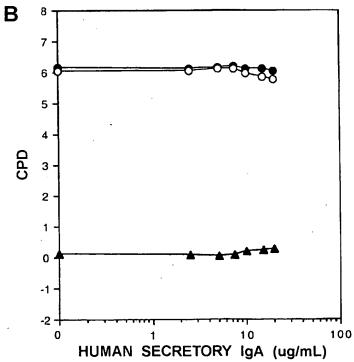
Contact: C.G. Mintz (713) 238-8000

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FIGURE 129

EFFECT OF HUMAN PLASMA IgA (A) AND SECRETORY
IgA (B) ON T47D CELL GROWTH IN SERUM-FREE MEDIUM





LEGEND: Closed circles = $+ E_2$ Open circles = $- E_2$

Closed triangles = Estrogenic effect

 Inventor: Sirbasku

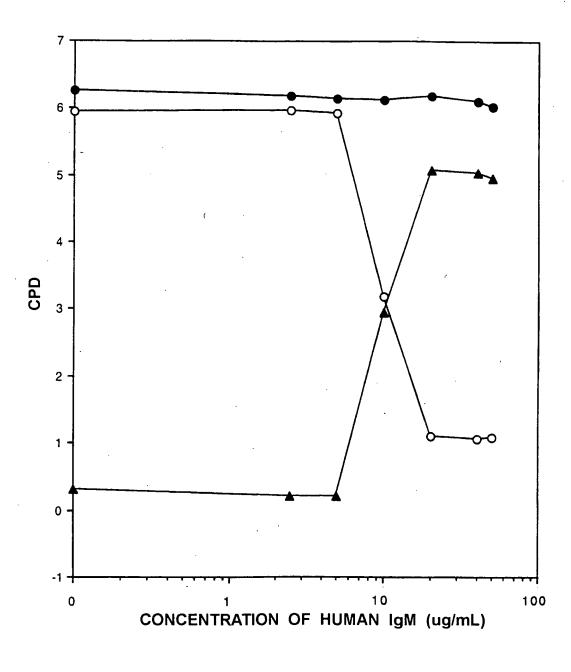
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FIGURE 130

EFFECT OF HUMAN IgM ON T47D CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

Closed circles = $+ E_2$

Open circles = $-E_2$

Closed triangles = Estrogenic effect

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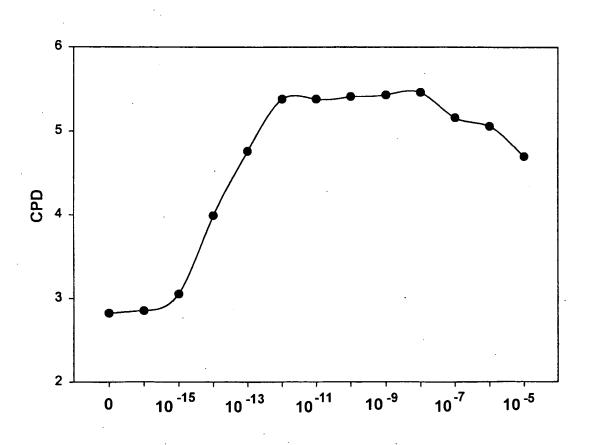
Express Mail EL818623541US Inventor: Sirbasku

Atty Dkt. No. 1944-00201 Contact: C.G. Mintz (713) 238-8000

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FIGURE 131

EFFECT OF ESTRADIOL ON T47D CELL GROWTH IN SERUM-FREE MEDIUM WITH 40 ug/mL HUMAN IgM



CONCENTRATION OF ESTRADIOL (M)

Inventor: Sirbasku

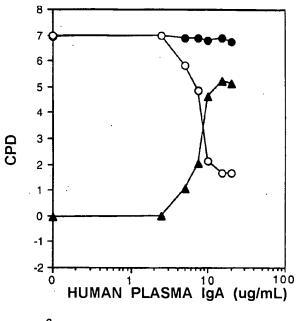
Atty Dkt. No. 1944-00201

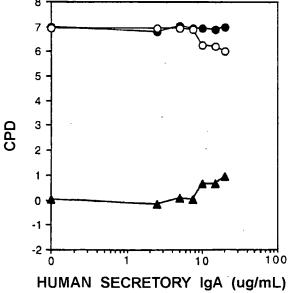
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FIGURE 132

EFFECT OF HUMAN PLASMA IGA (A) AND SECRETORY IGA (B) ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM





LEGEND: Closed circles = + E₂

Open circles = - E₂

Closed triangles = Estrogenic effect

Inventor: Sirbasku

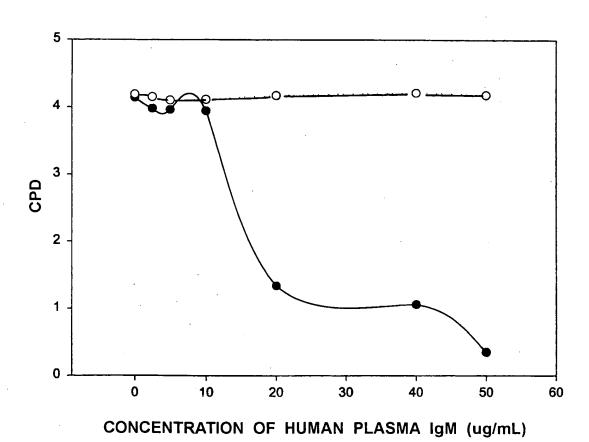
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Contact: C.G. Mintz (713) 238-8000

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FIGURE 133

EFFECT OF HUMAN PLASMA IgM ON ZR-75-1 CELL GROWTH IN SERUM-FREE MEDIUM



LEGEND:

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Inventor: Sirbasku

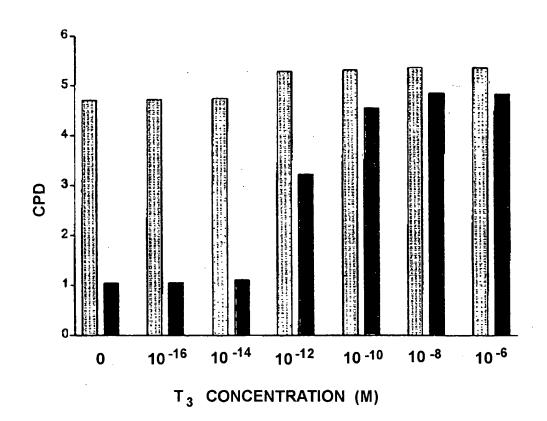
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EFFECT OF HUMAN IgM ON HT-29 CELL GROWTH IN THE PRESENCE OF INCREASING CONCENTRATIONS OF $\rm T_3$



LEGEND:

= T₃ Titration

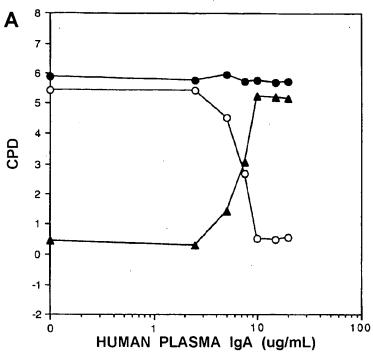
= T₃ Titration + 40 ug/mL lgM

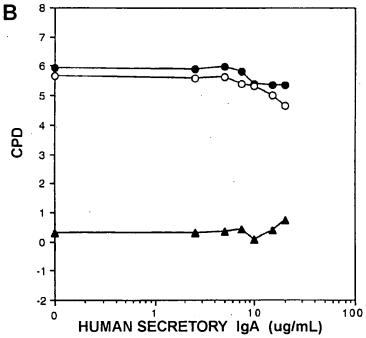
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EFFECT OF HUMAN PLASMA IGA (A) AND SECRETORY IgA (B) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM





LEGEND: Closed circles = $+ E_2$ Open circles = $-E_2$

Closed triangles = Estrogenic effect

Inventor: Sirbasku

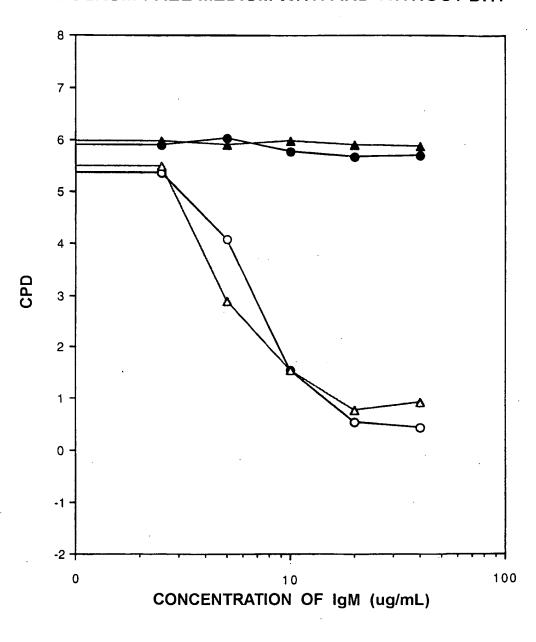
Atty Dkt. No. 1944-00201

Contact: C.G. Mintz (713) 238-8000

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FIGURE 136

EFFECTS OF HUMAN PLASMA IGM VS IGM DERIVED FROM MYELOMA CELLS ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM WITH AND WITHOUT DHT



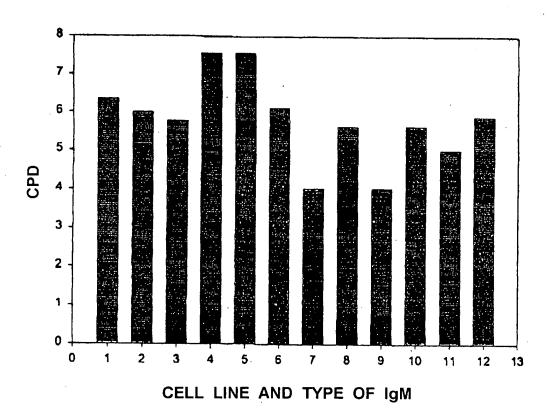
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FIGURE 137

ESTROGENIC EFFECT OF 50 ug/mL OF VARIOUS IgM'S ON SEVERAL DIFFERENT CELL LINES



- 1. Human IgM on MTW9/PL2 Cells = 6.36 cpd
- 2. Mouse IgM on MTW9/PL2 Cells = 6.00 cpd
- 3. Rat IgM on MTW9/PL2 Cells = 5.77 cpd
- 4. Human IgM on H301 Cells = 7.57 cpd
- 5. Mouse IgM on H301 Cells = 7.56 cpd
- 6. Rat lgM on H301 Cells = 6.11 cpd
- 7. Human IgM on GH1 Cells = 4.12 cpd
- 8. Rat IgM on GH1 Cells = 5.83 cpd
- 9. Human IgM on GH3 Cells = 4.09 cpd
- 10. Human IgM on GH4 Cells = 5.41 cpd
- 11. Human IgM on MCF-7A Cells = 5.01 cpd
- 12. Human IgM on MCF-7K Cells = 5.89 cpd

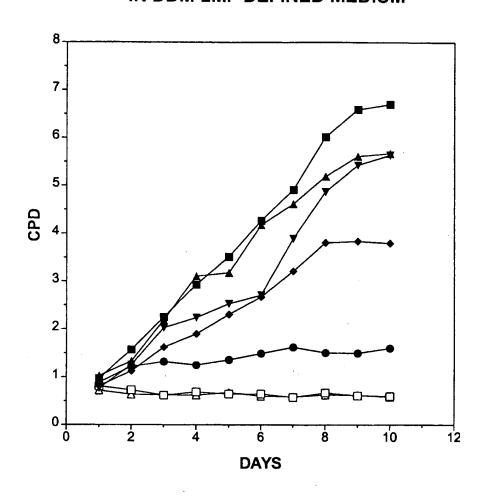
Inventor: Sirbasku

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FIGURE 138

EFFECT OF TAMOXIFEN ON T47D CELL GROWTH IN DDM-2MF DEFINED MEDIUM



LEGEND:
$$\rightarrow$$
 SFM + E₂

$$\rightarrow$$
 SFM - E₂

$$\rightarrow$$
 SFM + 10⁻⁹ M TAM
$$\rightarrow$$
 SFM + 10⁻⁸ M TAM
$$\rightarrow$$
 SFM + 10⁻⁷ M TAM
$$\rightarrow$$
 SFM + 10⁻⁶ M TAM
$$\rightarrow$$
 SFM + 10⁻⁵ M TAM

Inventor: Sirbasku

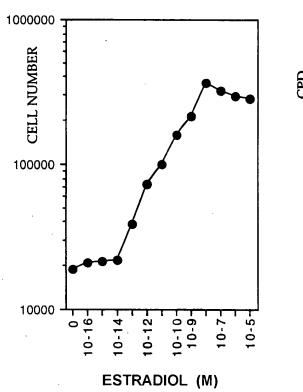
Atty Dkt. No. 1944-00201

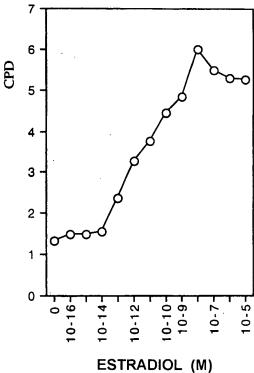
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FIGURE 139

EFFECT OF INCREASING ESTRADIOL CONCENTRATIONS ON T47D CELL GROWTH IN SERUM-FREE AND PHENOL- RED FREE MEDIUM WITH 10⁻⁷ TAMOXIFEN





NOTE:

DATA ARE EXPRESSED AS BOTH CELL NUMBER AND CPD

Inv

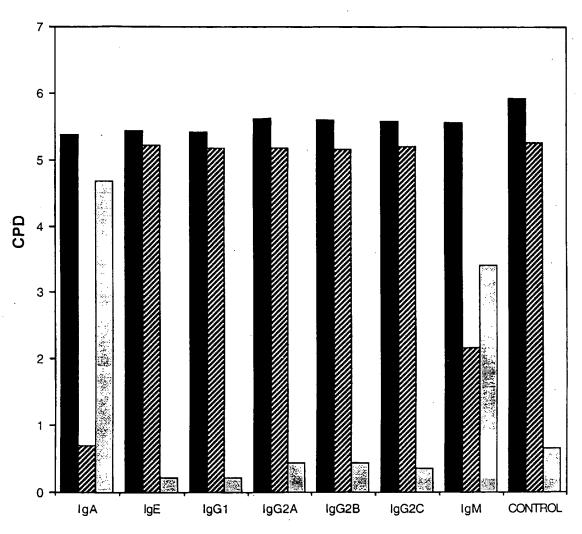
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FIGURE 140

EFFECT OF RAT IMMUNOGLOBULINS ON MTW9/PL2 CELL GROWTH IN SERUM-FREE MEDIUM



CONCENTRATION OF RAT IMMUNOGLOBULINS (15 ug/mL)

LEGEND:

■ = Estrogenic effect

CONTROL IS SERUM-FREE MEDIUM ALONE ± E2

सम्प्रमानस्य सम्प्रा पाइम्प सरम्पर प्राप्त सम्प्रमानस्य सम्प्रमानस्य स्थापन्त स्थापन्त स्थापन्त स्थापन्त स्थाप |- स्थापी सम्प्री मूल्यो मूल्या सम्प्रमानस्य स्थापने स्थापने सम्पर्धित स्थापी सम्प्री मूल्या

Inventor: Sirbasku

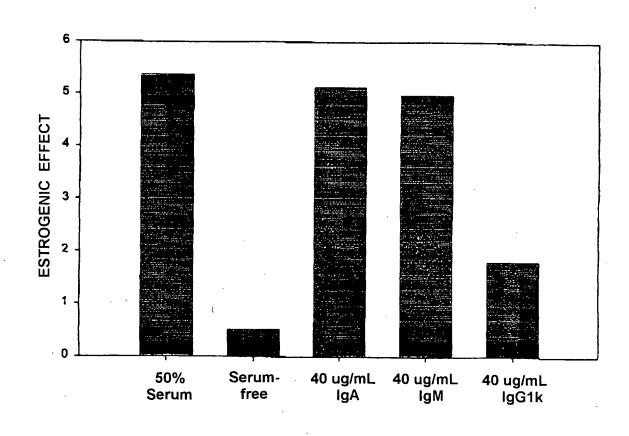
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FIGURE 141

ESTROGENIC EFFECT GENERATED BY IMMUNOGLOBULINS WITH T47D CELLS IN SERUM-FREE MEDIUM



IMMUNOGLOBULIN ADDED

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Inventor: Sirbasku

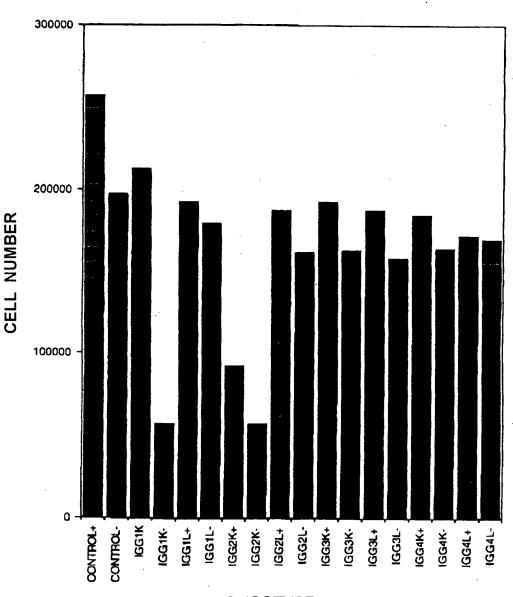
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FIGURE 142

EFFECT OF IgG ISOTYPES (40 ug/mL) ON LNCaP CELL GROWTH IN SERUM-FREE MEDIUM



IgG ISOTYPE ADDED

LEGEND:

= DHT Added

- = No DHT Added



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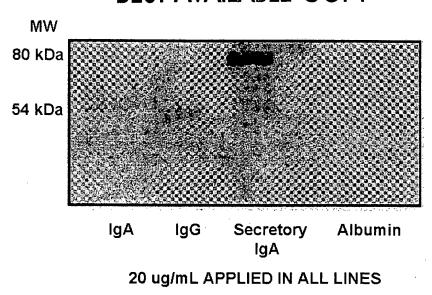
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FIGURE 143

DETECTION OF SECRETORY COMPONENT IN SECRETORY IGA WITH ANTI-SC ANTIBODY

BEST AVAILABLE COPY



IgA = Human Plasma

IgG = Human Plasma

Secretory IgA = IgA from Milk

Albumin = Human

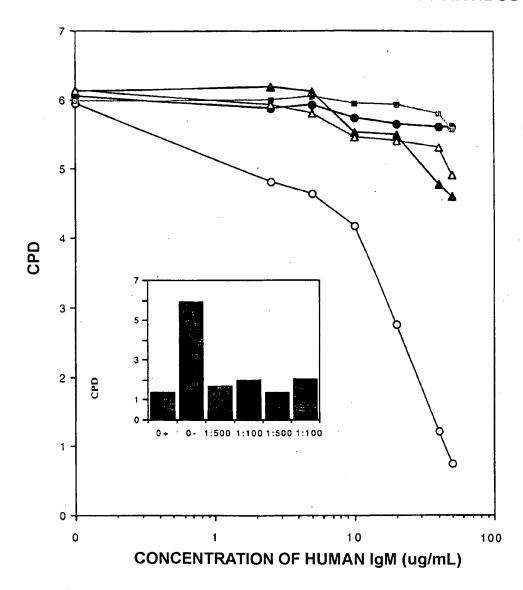
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Contact: C.G. Mintz (713) 238-8000

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HUMAN IgM TITRATION ON T47D CELLS GROWN IN SERUM-FREE MEDIUM WITH DIFFERENT DILUTIONS OF ANTI-SC ANTIBODY



INSERT: EFFECT OF RABBIT SERUM ON T47D CELLS INCUBATED WITH 40 ug/mL HUMAN IgM

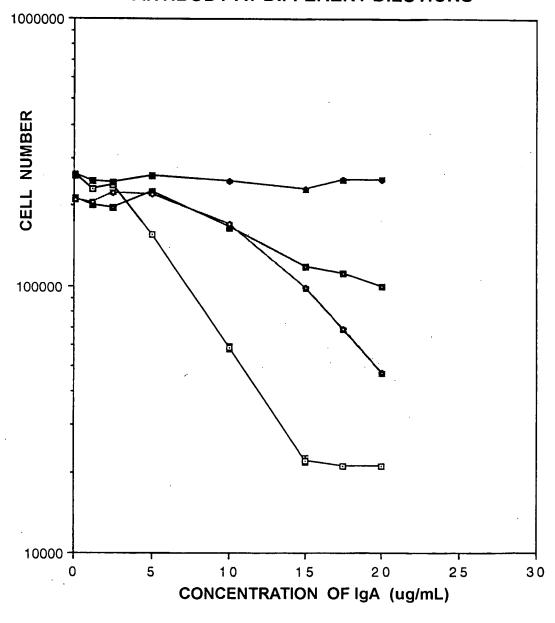
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FIGURE 145

EFFECT OF IgA ON LNCaP GROWTH IN THE PRESENCE OF ANTI-SECRETORY COMPONENT ANTIBODY AT DIFFERENT DILUTIONS



LEGEND: $-\omega$ = Control

→ = 1:100 Dilution of Anti-SC Antibody - = 1:500 Dilution of Anti-SC Antibody

= 1:1000 Dilution of Anti-SC Antibody



Inventor: Sirbasku

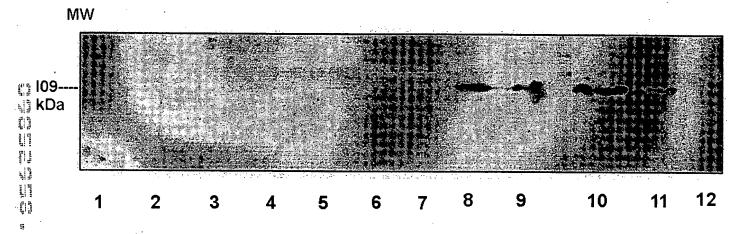
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FIGURE 146

WESTERN BLOT: ANTI-SECRETORY COMPONENT BEST AVAILABLE COPY



- 1. MW
- 2. ALVA 41: 40 ug
- 3. ALVA 41: 20 ug
- 4. DU 145: 40 ug
- 5. DU 145: 20 ug
- 6. HUMAN FIBROBLAST: 40 ug
- 7. HUMAN FIBROBLAST: 20 ug
- 8. LNCaP: 40 ug
- 9. LNCaP: 20 ug
- 10. MDCK1: 20 ug
- 11. MDCK1: 10 ug
- 12. PC3: 40 ug



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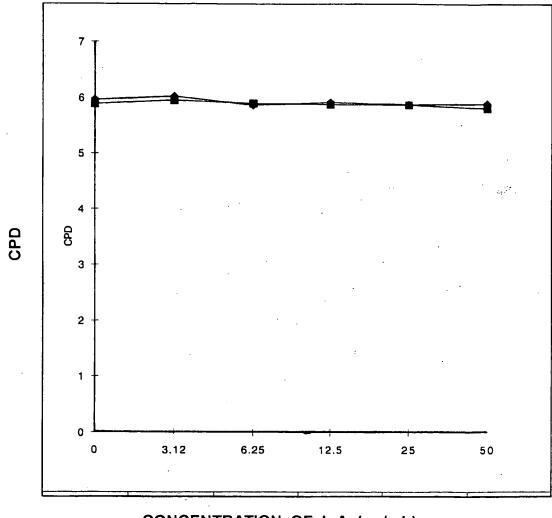
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FIGURE 147

EFFECT OF HUMAN PLASMA IGA ON DU145 CELL GROWTH WITH AND WITHOUT DHT



CONCENTRATION OF IgA (ug/mL)



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FIGURE 148

EFFECT OF HUMAN PLASMA IGA ON PC3 CELL GROWTH WITH AND WITHOUT DHT

